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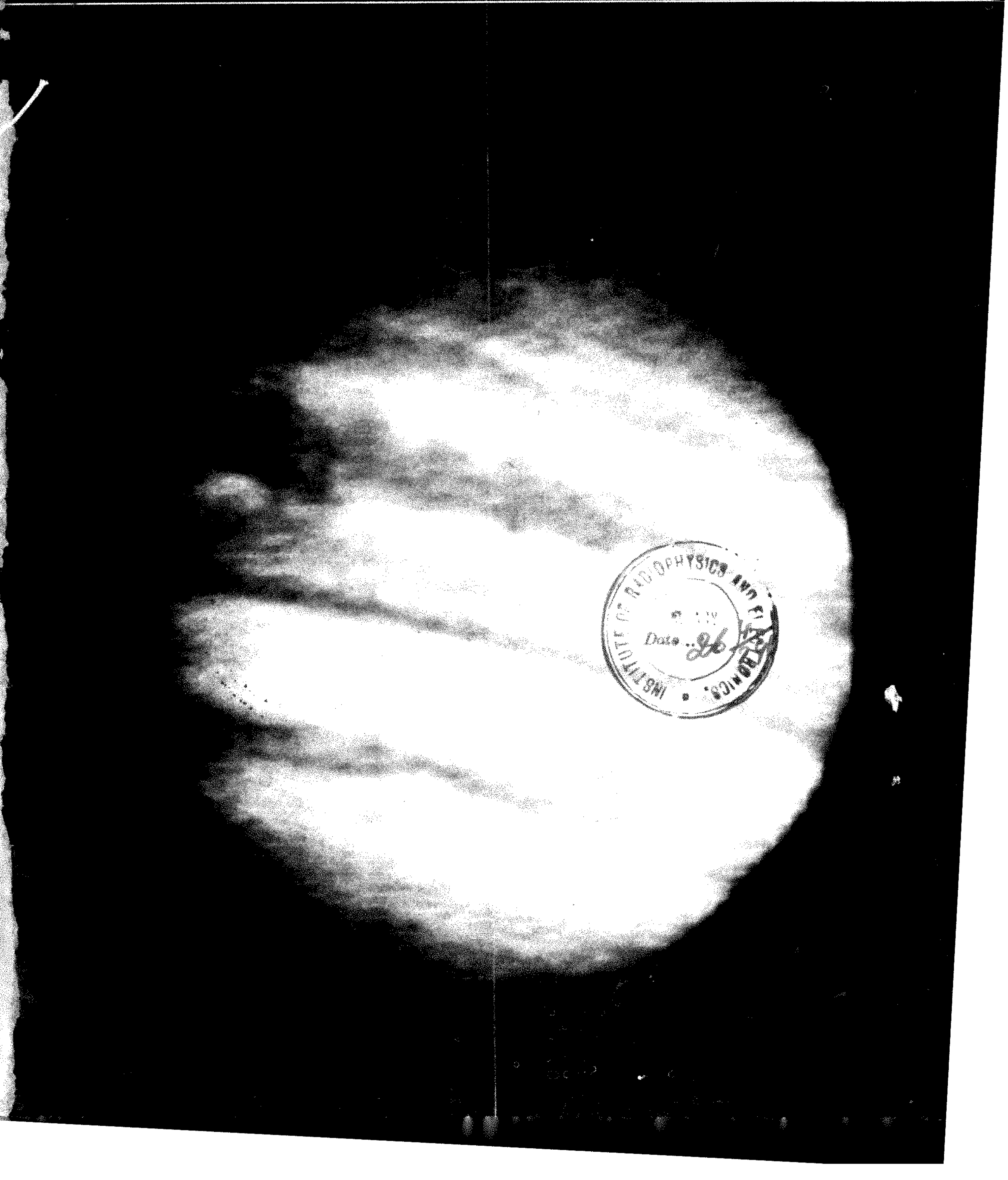

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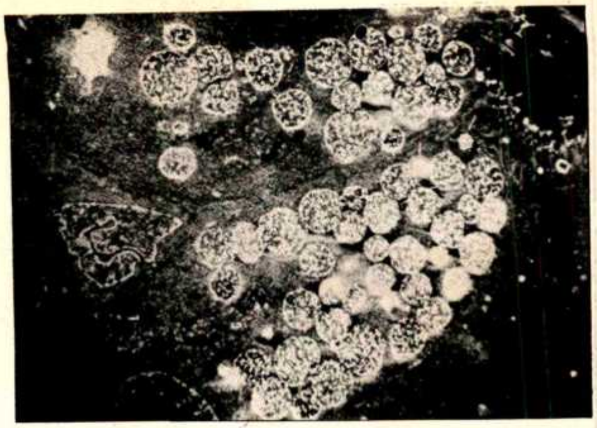

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Pioneer 10 picture of Jupiter, showing banded cloud structure and Great Red Spot. See also page 5.

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A New Year –
A New Journal: Cell

January 1st was not only the first day of a new year—but also the publication date of the first issue of **Cell**, a new monthly journal of cell and viral biology from The MIT Press. Edited by Benjamin Lewin, formerly the editor of *Nature New Biology*, **Cell** provides a wide-range journal publishing articles of general interest in molecular biology, cell biology, tumor virology and immunology, genetic development and other related areas. Papers are refereed as rapidly as possible and those accepted will be published within twelve weeks.

Cell also includes an extensive review section. In addition to those reviews with the traditional role of discussing a field in some depth, short reviews give conceptual accounts of recent advances in areas of cellular biology, for the benefit of researchers in other areas. Book reviews and discussions of meetings are also included.

The January issue of Cell includes the following articles:

Genetically directed preferential X-activation seen in mice: U. Drews, S. R. Blecher, D. A. Owen and S. Ohno

Ouabain-resistant mutants of mouse and hamster cells in culture: R. M. Baker, D. M. Brunette, R. Mankovitz, L. H. Thompson, G. F. Whitmore, L. Siminovitch and J. E. Till

A uridine kinase-deficient mutant of 3T3 and a selective method for cells containing the enzyme: L. Medrano and H. Green

SpoT, a new genetic locus involved in the stringent response in E. coli: T. Laffler and J. Gallant

The messenger-like poly-A containing RNA species from the mitochondria of mammals and insects: M. Hirsch, A. Spradling and S. Penman

Existence of methylated messenger RNA in mouse L cells: R. P. Perry and D. E. Kelley

Arrangement of specific oligonucleotides within poly-A terminated Hn RNA molecules: G. R. Molloy, W. R. Jelinek, M. Salditt and J. E. Darnell

Mixed splenocyte cultures and graft versus host reactions selectively induce an "S-tropic" murine type C virus: C. J. Sherr, M. M. Lieber and G. J. Todaro

Studies on the levels of cyclic AMP in cells transformed by wild type and temperature sensitive Kirsten sarcoma virus: R. A. Carchman, G. S. Johnson, I. Pastan and E. M. Scolnick

The February issue of Cell will include the following articles and reviews:

Viral Hepatitis, the B Antigen and Liver Cancer: A. J. Zuckerman

Integration and Excision of Phage Lambda: M. Gottesman

GUG and UUG are Initiation Codons In Vivo: J. H. Miller

The Molecular Biology of Tumour Viruses, edited by John Tooze: B. Mahy

Altered Metabolism of the Guanosine Tetraphosphate, ppGpp, in Mutants of E. coli: G. Stamminger and R. A. Lazzarini

Continuous Production of Radiation Leukemia Virus in C57BL Thymoma Tissue Culture Lines: Purification of the Leukemogenic Virus: M. Haas

Changes in RNA in Relation to Growth of the Fibroblast. I. Amounts of mRNA, rRNA and tRNA in Resting and Growing cells: L. Johnson, H. Abelson, H. Green and S. Penman

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Guide to authors

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- Articles are up to 3,000 words in length with at most six figures and may either be reports of major research developments in a subject or broader reviews of progress.
- Letters are brief reports of research of unusual and wide interest, not in general longer than 1,000 words; at most they have three or four figures.
- 'Matters Arising' permits occasional short discussion of papers that have previously appeared in *Nature*. A limit of 350 words is placed on contributions in this category.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the Système Internationale. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible. exp (a) is preferred to e^a if 'a' is more than one character. Articles should be accompanied by an abstract of not more than fifty words, and the abstract should list the main conclusions that are drawn.

References are indicated by superscripts in the text. The style may be gleaned from any contemporary *Nature* with the following two changes:

(i) If it is necessary to refer to several references by the same author at once, only one reference number need be given.

(ii) The last page as well as the first of any reference should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963–65). 'Personal communication' and 'unpublished work' should be incorporated in the text.

Artwork should be sent with the manuscript. All artwork should be marked with the author's name. Line drawings should preferably be in Indian ink on heavy cartridge paper, although other materials are acceptable; thin, shiny, folded, torn or heavily handled material should be avoided. Matt rather than glossy photographs are preferred. Figures are usually reduced to one column width. The originals should be about twice their final size. Figures, particularly maps, should contain nothing but essential material. It is preferred that the original be unlabelled, but with a copy containing lettering. Labelling on photographs should if possible be avoided entirely.

A fuller guide appeared in *Nature* (**246**, 238; 1973).

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Recent Scientific and Technical Books v

The appearance of *Nature* in a once-a-week format coincides with severe restrictions on industrial use of power. This number is thus, we regret, reduced to ninety-six pages in length. Arrangements have been made to publish the journal weekly during the period of stringency, but the January 11 issue has had to be cut to sixty-four pages. We hope that *Nature* will grow back to its planned size shortly thereafter. Delays in despatch, other than those introduced by the post, are not expected.

The *Nature* logo on the front cover and elsewhere is basically Baskerville Old Face. This was designed by John Baskerville (1706–75) who introduced several new elements into eighteenth century lettering, although his types were not put to wide commercial use until 1923. The rest of the journal is set in members of the Times family—Bold and Semi-bold for headings and New Roman for the text.

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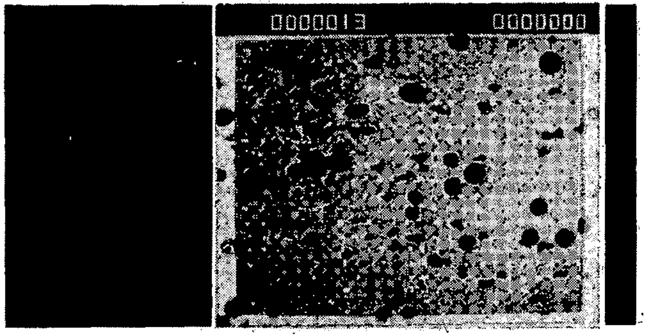

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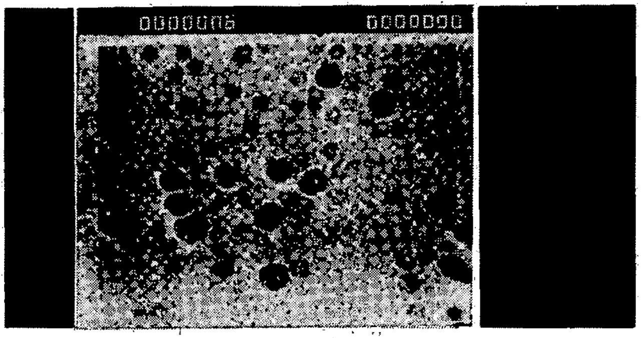

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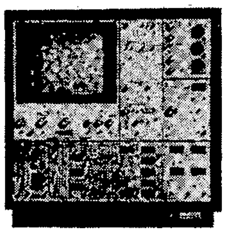

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Volume 247 January 4, 1974

Government by stealth and secrecy

BRITAIN is now confronted with a severe economic crisis, the ingredients of which are well known, and even when the miners, engine drivers and power station engineers return to full-time working there is bound to be a period of general inconvenience and little prospect of improvements in the standard of living for some time to come. Many things are bound to change as a result of the crisis and it is to be hoped that among them will be the style of government under which Britain has operated for a very long time—specifically the obsessive secrecy and stealth with which Whitehall works and the serious lack of information provided to the public.

This complaint is, of course, fashionable and it is equally fashionable to pay lip-service, as did Mr Heath in 1970, to 'open government'. The question is not whether Britain provides less information than other countries, although it palpably does in comparison with, say, the United States, but whether it provides enough information for those who wish to know, and who do not choose to work for the Civil Service, to be able to come to independent and balanced conclusions.

A conventional defence is to point to such institutions as Question Time in the House of Commons and a free press. This is a very shallow response. The character of Question Time is to find quick, witty and crushing responses to issues raised. This is a political process and success at this facile operation is deemed to be a prerequisite of a good politician. This highly prized skill with words, which spills over into all political exchange, does nothing but damage to a country which needs rational assessment. It is no accident that 'evaluation', a word used almost to excess in the United States, is relatively rare in Britain.

Can a free press make up for this deficiency? Journalists have to depend on a heterogeneous collection of sources for information, from leaks to plain inference. It is simply not possible always to guarantee that one man working against a system will be successful, and it would be foolish to expect more than honest effort. A free press is no replacement for free information and no journalist would regard an increase in the flow of facts as other than beneficial to his operations, certainly not as a threat to his profession.

During the energy crisis the government has operated sufficiently frequently by stealth and suppression of information to raise serious doubts that its style can be supported indefinitely by an electorate. After weeks of denying that the fuel shortage was serious enough to warrant strong corrective action, ration books are issued with such indecent haste that the As and Bs with coughs and colds have probably missed out. After Post Offices close for the weekend nine days before Christmas an announcement is made that no further Christmas parcels will be accepted. After talking of still going for growth, in the full knowledge of fuel shortages, the government days later imposes a three-day working week and demolishes thoughts of growth.

This is rule by edict and secrecy and is not, it must be emphasised, restricted to the present government. Rather, the channels for advance warning, discussion and compromise fail to exist.

There is much talk at present of the appointment of an energy supremo in Britain, and several names have been mentioned. It is difficult to know whether the idea can be welcomed. If the supremo is a party politician dedicated to the present system of operation and only prepared to give political answers to questions, then the creation of another agency may profit the country not at all. If, on the other hand, someone can be found who will work honestly and openly and recognise the need for the public to receive facts and figures in support of decisions he will deserve wide support.

100 years ago

ON TEMPERATURE CYCLES

SINCE the discovery of an eleven years' period in the phenomena of solar spots, several corresponding periods (it is now well known) have been demonstrated in terrestrial phenomena, more especially in those of magnetism, auroras, cyclones, and rainfall. With regard to weather changes, it has been thought by Dove, that the tracking of a cycle in these could not, theoretically, be made an object of research ; and that while some indications of a periodicity might appear, a great part of the complicated changes named must be, from the nature of the case, quite unperiodical. The series of observations by Dove on the subject led him to the conclusion (1) that divergences from the normal, especially those of temperature, are not local, but spread over large surfaces ; but (2) that negative divergences, in one region of the earth, are compensated by positive in another ; and conversely. That the compensation is perfect, and that the quantity of heat annually given by the sun is constant, has been affirmed also by Maury and others.

From Nature, 9, 184, January 8, 1874.

news and views

Global dissemination of hepatitis B virus

INFECTION with the virus, or at least one of the viruses, of hepatitis type B is associated with the appearance in the serum of a specific antigen previously referred to as Australia antigen. Hepatitis B antigen has been found to possess a common group specific surface antigen *a* and at least two principal subdeterminants *d* and *y*, which behave in a mutually exclusive manner although carried on the same antigen particle. Transmission experiments to human volunteers and the common observation of only one subtype in localised outbreaks of hepatitis B infection suggest that the subtypes are the phenotypic expression of distinct genotypes of the virus and are not determined by the host (Le Bouvier, *Am. J. Dis. Child.*, **123**, 420; 1972). Bancroft and his associates (*J. Immunol.*, **109**, 842; 1972) first detected two additional antigenic subdeterminants, named *w* and *r*, and the following phenotypes of hepatitis B virus were recognised: *adw*, *adr* and *ayw*. The missing fourth phenotype *ayr*, which seems to be uncommon, has since been described in the Far East. A remarkable geographical pattern of distribution of hepatitis B antigen subtypes is now emerging (see *Nature new Biol.*, **242**, 34; 1973) with four global zones, where there is an excess of one subtype, and regions where a mixture of subtypes is common.

Diversity of subtypes

Recent studies illustrate this geographical diversity. The high proportion of immigrants in Israel from all over the world provides an almost unique variety of ethnic and geographical background. Approximately half of the blood donors in Israel are foreign-born and most of the others are first-generation Israeli-born Jews. Bar-Shany and her colleagues (*Vox Sang.*, **25**, 105; 1973) characterised 180 asymptomatic antigen-positive blood donors for subdeterminants *d* and *y*: 122 (78%) of 154 males and nine of twelve females carried *y*. Of sixty consecutively identified antigen carriers, selected initially according to their suitability for subtyping and subsequently for representation of dissimilar geographical background, forty-seven (78%) carried the subdeterminant *y* and thirteen (22%) carried *d*. Immigrants to Israel from North Africa, the Near and Middle East, Southern European countries and Southern Russia had almost exclusively subdeterminant *y*, in contrast to the predominance of *d* among blood donors living in North Central and North Europe and North America. Interestingly, Jewish immigrants from South Central Europe have an intermediate frequency of the *d*:*y* pair between North and South European donors.

Twenty-five hepatitis B antigen-positive samples, selected for suitability for subtyping and for geographical representation, were tested for subdeterminants *w* and *r*. All were found to carry *w*, despite variegated geographical origin. It would be reasonable to anticipate that at least some immigrants may have acquired their antigen carrier state after reaching Israel and therefore that the proportion of *y* may differ from carriers still resident in the countries of origin. Such a shift towards *y* might be expected especially in first-generation Israelis from high *d* prevalence areas, provided exposure at school or similar places commonly resulted in spread of hepatitis B virus. First-generation Israelis of Northern or Middle European background, however, had the same frequency of subdeterminant *d* as immigrants from these regions, suggesting the home as the important site of transmission.

These facts are in agreement with the view that close and/or prolonged contact is necessary for transmission by hepatitis B carriers. Feinman and his associates (*Lancet*, ii, 867; 1973) subtyped sixty-three symptom-free blood donors, who were found to carry hepatitis B antigen in Toronto: thirty carried subtype *adw*, eight *adr* and twenty-five *ayw*. When the geographical origin of the donors was investigated, the subtypes were found to be unevenly distributed. Most donors who were *adw* were born in Canada, China, Germany and the West Indies, whereas most of those who were *ayw* originated from Mediterranean countries. All eight donors who were *adr* were born in China. 139 family members of forty-six antigen-positive donors were also tested and eighteen (13%) first-degree relatives of nine index cases were found antigen-positive. Subtyping of the antigen in fourteen relatives showed that all had the same subtype as the index case.

The relationship of subtypes to the country of birth and the home rather than place of residence supports the concept that the carrier state of hepatitis B antigen is determined early in life. But even these studies may be an oversimplification of the problem. Soulier and Courouce-Pauty (*Vox Sang.*, **25**, 212; 1973) recently obtained results from subtyping of hepatitis B antigen, using unabsorbed or selectively absorbed antisera, which suggested the subdivision of antigens with the *ay* subdeterminants into three categories and the *ad* subtypes into two categories. These categories could not be explained in terms of the *w* or *r* subdeterminants since *w* was found in all five categories. The results can be explained, however, by postulating the existence of three subdeterminants of the common group specific determinant *a*, so that *a* is designated *a1*, *a2* and *a3*. Thus *ay* is classified as *a1y* (found in this study in 13% of apparently healthy Vietnamese carriers), *a2y* (found in most healthy French carriers and patients with antigen subtype *ay*) and *a3y* (mostly African carriers of *ay*). On the other hand, *ad* is subdivided into *a2d* (common *ad*) and *a3d* (apparently a very rare subtype).

The differences between the subspecificities of *a* may be both qualitative and quantitative, probably with overlapping and predominance between these three subdeterminants. As with the *d* and *y* subdeterminants *a1*, *a2* and *a3* are related to the virus and not to the host. In this study it was noted, for example, that whereas *a1* was found in Vietnamese, it may also be found in Caucasians after a stay in Vietnam; conversely, some Vietnamese living in France were found to carry *a2*. The same is true for *a2*, which is common in Caucasians (but not restricted to them) and, although *a3* seemed to be most common in African countries, it was also found in French Caucasians who became infected in Africa.

Geographical zones

The overall picture which is therefore emerging is a Y zone, with antigen phenotype *ayw*, incorporating the Middle East, Iran and Pakistan, the Eastern Mediterranean and Southern European countries and Africa (although Africa has not been adequately studied). The D zone, with phenotype *adw*, is centred in North Europe, North and Central America, and both *adw* and *adr* are prevalent in the Far East, Thailand, Malaysia, Indonesia and New Guinea. The R zone, predominantly *adr*, seems to be located in South East Asia and the Far East. The fourth region is the Pleotypic zone, centred in Oceania and particularly around Bougainville, as reported on page 38 of this issue of *Nature* by Mazzur and her colleagues. In this region practically all possible mixtures of the recognised phenotypes of hepatitis B antigen have been found.

Incursions of different subtypes into these zones and the establishment of mixed zones inevitably result from population movements. But since the subtypes are determined by the virus and not by the host, an epidemic of one strain of hepatitis B virus might result in a spate of that particular uniform strain at one time, and a later epidemic of a different subtype might leave carriers of another subtype even in the same area. This has been recorded in Stockholm, for example. Magnius and his associates (*Scand. J. infect. Dis.*, 5, 81; 1973) examined stored sera from seventy-two patients with viral hepatitis admitted to a hospital in Stockholm in 1953–54 and sera from 202 patients with hepatitis admitted in 1970. Hepatitis B antigen was detected in 43% and 49% of the patients respectively. The subtypes from these two groups and from ninety-five antigen-positive drug addicts were determined. Twenty-two (71%) of thirty-one patients from the 1953–54 group were of *ad* subtype whereas only two were *ay* (seven samples could not be typed). In 1970, however, seventy-five (76%) of ninety-nine patients were *ay* whereas only twelve were *ad* (the remaining twelve sera could not be subtyped). Ninety (95%) of the antigen-positive drug addicts were *ay* and five were *ad*. A shift has thus occurred in Stockholm since 1953–54 from predominantly *ad* to the *ay* subtype. It is speculated that the tourist influx from Northern Europe to the Southern Mediterranean countries, the Middle East and North Africa, an *ay* zone, as well as the attraction of these countries to drug addicts may have been factors in the shift of hepatitis B antigen subtypes noted in Stockholm.

The latest report by Mazzur and her associates in the current issue of *Nature* also confirms the varied geographical distribution of antigen subdeterminants *d*, *y* and *w*, and, although the antigen mapping of the world is fragmentary and far from complete, information is rapidly accumulating. Hepatitis B antigen has previously been shown to be a useful epidemiological marker; it may well prove to be a fascinating ecological marker of both ancient and more recent population migration.

From our Medical Virology
Correspondent

Plant diversity in grassland

from our Plant Ecology Correspondent

THE publication by the Kent Trust for Nature Conservation of papers given at a conference on chalk grassland management held a year ago at the King's College Field Centre, Rogate, Hampshire, will reopen the question of what determines plant species diversity in this habitat (see *Nature*, **240**, 443; 1972). It may also raise new questions regarding the objectives of management practices and priorities in conservation.

The diversity issue has been clarified by Grime (*Nature*, **242**, 344; 1973), who has come to the conclusion that diversity in grassland habitats is clearly related to environmental stresses. If stress is low, then grasslands become dominated by species of high physical competitive ability, that is species which grow rapidly, are robust and produce dense litter. Stress reduces the performance of these species, allowing other species to enter the community as a result of the lowering of competition. Diversity thus increases with stress until the stress becomes so severe that only species which are specifically adapted to that particular adverse factor are capable of survival. Then diversity falls. Some authors emphasise the role of predation in the maintenance of vegetation diversity (for example, Harper, *Brookhaven Symp. Biol.*, **22**, 48; 1969) whereas others feel that nutrient availability may be the critical factor (for example, Green, *Biol. Conserv.*, **4**, 378; 1972).

In the recently published report (*Chalk Grassland*, ed. by Jermy and Stott, Kent Trust for Nature Conservation; 1973), both sides of the argument are represented. Wells considers that the composition of a grassland community can best be understood by reference to the respective life forms and growth periods of its constituent species. In other words, if mowing or grazing stresses are applied to a grassland then the species which lose the smallest proportion of their above ground biomass will be at a selective advantage. Tall, robust species are eliminated to the advantage of short-growing species, thus allowing the development of a diverse assemblage of species with an average 67% of low-growing hemicrytophytes.

Harding defends the point of view that nutrient availability limits the growth of aggressive species. Using Shannon's diversity index, he demonstrates a negative correlation between angiosperm diversity in the grass sward and total nitrogen in the soil. He follows Green in the belief that a build-up of nitrogen in the soil permits invasion and attainment of dominance by robust species, thus reducing overall diversity. It is always dangerous, however, to infer causation from correlation. One could equally well argue that the presence of robust, dominant species causes a build-up of nitrogen in the soil by rapid litter production and hence the encouragement of soil microbiol activity, including nitrogen fixation. Suspension of robust species by applying a grazing stress could keep soil nitrogen levels low. The nutrient argument still lacks direct experimental support.

The quest of conservationists for biotic diversity becomes even more complex when one considers the zoological aspect of the problem. For invertebrates in particular, microclimatic diversity (which is a function of vegetation structural complexity) is an overriding influence upon the potential faunal diversity. It is not surprising, therefore, that Morris demonstrates a positive correlation between the number of species of *Auchenorhyncha* and the vegetation height at the sampling sites. It does, however, pose a management problem, since in selecting a management regime which determines vegetation height, one must choose between floral and faunal diversity.

Proteins and odour perception in insects

from our Insect Physiology Correspondent

THERE have been several suggestions that enzymatic processes are involved in olfactory perception in insects. Schneider observed that the antennae of the male silkworm rapidly degrade the sex pheromone bombycol following stimulation; and Kasan and Kaissling confirmed that bombycol, (E)-10-(Z)-12-hexadecadien-1-ol, was progressively degraded into acidic and ester fractions by enzymes in the antennae and other parts of the body. In this way the receptors quickly become competent once more to receive the olfactory stimulus. Norris, Ferkovich and others have shown that naphthoquinones, which function as feeding inhibitors in the cockroach *Periplaneta*, exert their activity by binding to sulphydryl groups of specific antennal proteins.

Ferkovich, Mayer and Rutter (*J. Insect Physiol.*, **19**, 2231; 1973) have now studied the sex attractant of the cabbage looper, *Trichoplusia ni*, along the same lines. This pheromone is (Z)-7-dodecen-1-ol acetate. When exposed to a soluble protein present in the male antennae it suffers enzymatic conversion to the alcohol (Z)-7-dodecen-1-ol—which not only lacks sex attractancy but is a potent inhibitor of behavioural responses to the normal pheromone. The protein responsible for enzymatic hydrolysis of the pheromone exists in smaller amounts elsewhere in the body, but it is most active in the antennae. The inhibitory alcohol also reacts, non-enzymatically, with the antennal protein; and both these interactions seem to be necessary for olfactory perception by the cabbage looper.

The close analogies between olfaction and juvenile hormone activity have been pointed out (*J. Insect Physiol.*, **15**, 73; 1969). The hormone likewise must be rapidly inactivated if it is to be kept under control; and the chief mode of inactivation is by means of an esterase which hydrolyses the terpenoid ester to the carboxylic acid, which is devoid of hormonal activity.

KCN and photosystem one

from our Photosynthesis Correspondent

THERE now seems to be no doubt that KCN inhibits photosynthesis by specifically blocking electron flow through photosystem one (S1). It has long been recognised that KCN will inhibit photosynthesis but its mode and site of action have not been clearly understood. Izawa *et al.* (*Biochim. biophys. Acta*, **314**, 328; 1973) now convincingly demonstrate that this compound inhibits electron transfer between cytochrome *f* (cyt *f*) and P700.

Izawa *et al.* used differential spectrophotometry and electron paramagnetic resonance (EPR) to monitor changes in the redox states of these two components which are closely associated with S1 activity. Oxidation of the S1 reaction centre can be detected either by an absorbancy change at 700 nm (for this reason it is called P700) or as an EPR signal with a *g* value of 2.0025. Cyt *f* acts as an electron donor to P700 and is oxidised by light preferentially absorbed by S1 (S1 light). When photosystem two (S2) light is superimposed on S1 light the oxidation levels of cyt *f* and P700 are reduced. These were classical observations first made in Duysen's laboratory in Holland some years ago which clearly demonstrated that S1 and S2 act in series and that cyt *f* and P700 are on the reducing side of the S2 water splitting reaction centre. As expected it was found that in the presence of 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU), a compound which blocks electron flow from S2 to S1, the reduction of oxidised cyt *f* and P700 by S2 light was inhibited.

Izawa *et al.* were able to demonstrate these properties in their spinach chloroplast preparations, but when the chloroplasts were incubated with KCN, S1 light only photo-oxidised P700 and not cyt *f*. Moreover, when cyt *f* was chemically oxidised the action of S2 light was to reverse this oxidation. On the other hand KCN severely restricted the net flow of electrons from S2 to photo-oxidised P700. These observations indicate without doubt that KCN acts by blocking electron flow between cyt *f* and P700. Izawa and colleagues argue that KCN interacts with plastocyanin, as suggested from *in vitro* experiments, and that this copper containing protein is the intermediate electron carrier between cyt *f* and P700.

Their general ideas have additional support from experiments involving the use of the artificial electron doning agents, diaminodurene, reduced 2.6-dichlorophenol indophenol and reduced N-methylphenazonium methosulphate (PMS) all of which bring about the reduction of oxidised cyt *f* and P700 in DCMU insensitive reactions. It was found with these artificial systems that KCN usually inhibited the reduction of P700 but not cyt *f* although leak past the KCN block was obtained in some conditions such as high PMS concentrations (>30 μM). Unlike normal electron flow this component did not support photophosphorylation.

This work of Izawa *et al.* not only gives good support to an old argument that cyt *f* is not the primary electron donor to S1 but also demonstrates that KCN can be used as a new and powerful tool for investigating S1 reactions in isolated coupled chloroplasts.

Satellites advance geodetic research

from a Correspondent

UNDER the auspices of the International Association of Geodesy, an important symposium on the Earth's gravitational field and secular variations in position was held in Sydney from November 26 to 30.

Through the Earth and Ocean Physics Applications Project (EOPAP) of NASA, the GEOS-C is due to be launched in 1974, and will utilise many of the techniques evolved through the ranging reflectors placed on the Moon by the Apollo astronauts. Many of the papers presented at the symposium described techniques for use with such satellites as elevated targets for ranging with pulsed lasers. The astonishingly high accuracy possible with these new methods will shortly become standard expectations in surveying and in geophysical and oceanographic research.

In an invited presentation, P. V. Angus-Leppan (University of New South Wales) assessed the future prospects for "four-dimensional geodesy", a term which describes the new high-precision techniques for providing position and gravity, and their changes within a short time span. He pointed out that the accuracies of one part in 10^8, which are being approached in these measurements, will shortly allow direct measurements of the velocity of movements of continental plates; from geological inference these plates are currently believed to be moving at a rate of about a few centimetres per year. To achieve the required accuracy, a network of observing stations must be set up on a global scale with international coordination. Angus-Leppan said that each station would constitute the fundamental geodetic observatory for its region and it would include, in a set of comprehensive facilities, laser ranging systems to both the Moon and artificial satellites, an antenna for interconnecting stations by very long baseline interferometry (VLBI), Doppler satellite instruments and a base for absolute gravity measurements.

Progress in the VLBI technique was discussed by several contributors. P. F. MacDoran (Jet Propulsion Laboratory, California Institute of Technology) reported that observations of intense extragalactic radio sources (notably QSOs) with two separated dish antennas operating at S-band wavelengths (13 cm) gave accuracies of a few centimetres for three-dimensional surveying over short separations of 16 km. In addition to measuring variations in universal time, intercontinental baselines (8,400 km) have been measured in VLBI tests between Goldstone, California, and Madrid, Spain. MacDoran predicted that, if a transportable 9 m diameter dish antenna were operated in conjunction with a fixed dish, like the 64 m ones at Goldstone and Madrid, accuracies of a few centimetres in three-dimensional positions would be practical for separations of up to several hundred kilometres, using X-band wavelengths (4 cm).

The importance of accurately calibrated measurements of position and gravity with time was emphasised by the geophysically-minded delegates. These measurements lead to estimates of slow, yet important, lateral and vertical velocities of crustal plates. Direct tests will then be possible for recent theories for predicting earthquakes related to dilatancy of rocks. Four Americans (D. E. Smith, R. Kolenkiewicz, R. W. Agreen and P. J. Dunn) described SAFE which has recently started to use satellite ranging techniques for measuring the motion between two points 900 km apart on opposite sides of the San Andreas fault system in California. Over a 7-yr period, the experimenters believe they will determine the average relative motion of the two sides of the fault to an accuracy of about 5 mm yr^{-1}.

The geoid is defined as the equipotential surface of the Earth's gravitational field corresponding to 'mean sea level', but, as R. S. Mather (University of New South Wales) pointed out, geodetic levelling results and mean sea level (as defined by tide gauge readings) do not correspond. The 'mounds' in the sea, with amplitudes of up to 2 m and wavelengths as great as 8,000 km, produce a topography of the sea surface. This leads to problems in geodesy because of the reliance on mean sea level as a reference datum for elevation. Mather indicated ways of solving this problem by using the precise radar altimeters in the satellites of the forthcoming EOPAP project. If confirmed and when mapped on a global scale, the topography of the sea surface will provide information for predicting ocean currents—a potential boon for more economic navigation of commercial shipping.

J. D. Boulanger (Institute of Physics of the Earth, Soviet Academy of Sciences) tackled the complex problem of the observed variations in gravity with time, and the interpretation of these data in terms of, for example, tectonic processes occurring within the Earth's interior. Yet, according to Boulanger, significant data on this point are scanty. So he proposed an international programme for studying the precise variation of gravity with time throughout the world. He described a 10-yr programme being established in the Soviet Union with a network of gravimetric stations of high precision in the territory of Eastern Europe and the west of the Asiatic part of the Soviet Union.

The network will be based on Sèvres, near Paris. Sèvres has been chosen by the Russians because, Boulanger said, this is the only place where an absolute result for a rate of change of gravity with time has been obtained. The experimenter responsible for this result, A. Sakuma (Bureau Internationale des Poids et Mesures) described the determinations of absolute gravity made by his team; the precision of their measurements is almost one part in 10^9.

Pioneer 10 exceeds expectations

THE remarkable pictures of Jupiter received from Pioneer 10 are alone sufficient to ensure that the mission is remembered as a success, even without data from the other experiments on board the spacecraft. It will take some time for details of these data to emerge; but the simple fact that Pioneer 10 passed unscathed through Jupiter's magnetosphere is itself enough to require understanding of the planet's radiation belts to be rethought.

Most astronomers expected the spacecraft to suffer some damage when it encountered energetic particles trapped in the Jovian magnetosphere, and some suggested that the experiments and telemetry would be rendered completely useless. But a few voices suggesting the opposite view were raised, and two papers in the issue of *Science* dated December 7 (four days after Pioneer 10's closest approach to Jupiter) show the extremes of opinion which co-existed before the spacecraft's encounter with the planet. Stansberry and White describe (*Science*, **182**, 1020; 1973) a model of the trapping of electrons and protons from the solar wind by the magnetic field of Jupiter which implies very high particle densities in the radiation belts, reaching levels 100 times greater than those used in the design of Pioneer 10. "This indicates", they said in the paper, "that there is a significant chance of radiation damage to the spacecraft".

Clearly, they were wrong. And a clue to just how their calculations were in error comes from a study by Hess, Birmingham and Mead (*Science*, **182**, 1021; 1973) of the effect of Jupiter's satellites on the particle fluxes in certain parts of the Jovian magnetosphere. Three of the Galilean satellites, in particular, turn out to be very effective in limiting the fluxes of energetic protons and electrons diffusing inward. Ganymede, Europa and Io all produce "precipitous" drops in the flux, so that the average flux in the plane in which these satellites lie "should be about a factor of 100 less than . . . if there were no absorbing moons". Hess and colleagues suggest that "This may be enough to prevent serious radiation damage to the spacecraft", and it now looks as if they were right.

Jupiter's red spot, a shadow of the Moon Io and Jupiter's cloud structure are shown in this photograph taken on December 1 as Pioneer 10 was about 2,500,000 km (1,580,000 miles) from the planet.

The importance of this, of course, extends beyond the satisfaction of having an idea proved right. It seems clear from the success of Pioneer 10 that spacecraft can approach fairly close to Jupiter without being severely damaged, and the presence of a satisfactory theory to account for the low particle fluxes encountered by the spacecraft suggests that similar low flux conditions will be encountered by other spacecraft following suitable trajectories. The need for close encounters is two-fold: first, it enables spacecraft to take an advantage of Jupiter's gravity in directing them on to Saturn and the other gas giants; second, with the present state of the art a Jupiter rendezvous mission, leaving a spacecraft in orbit around the planet, can only be achieved by firing the spacecraft's motors when it is deep in the Jovian gravitational well. So Pioneer 10 has lived up to its name by showing the way for future missions to the outer reaches of the Solar System.

Code of practice for radiation protection

from a Correspondent

A MEETING in London on November 14 was arranged jointly by the British Institute of Radiology and the Hospital Physicists' Association to provide a forum for discussion of the requirements and implementation of the 1972 revision of the Code of Practice for the Protection of Persons against Ionising Radiations arising from Medical and Dental Use.

The morning session was concerned with the main provisions of the new code and difficulties in its interpretation. J. Cole (Dudley Road Hospital, Birmingham) reviewed the steps leading to the present revision following the publication of the first edition of the code in 1957. He pointed out that the basic policy in formulating the code was "to set out the basic principles, and to give general guidance on good practice".

H. Miller (University of Sheffield) described the changes in the scope of the code and the general protection measures required. He said that the wording of the new code was more precise and the conditions for both essential and desirable requirements had been clarified.

G. M. Ardran (University of Oxford) and S. K. Stephenson (Christie Hospital, Manchester) then outlined the changes in the code relating respectively to the medical and physical aspects of diagnostic radiology. The relative responsibilities of the clinician and radiologist in reducing patient exposure were mentioned in relation to the problems of implementing the '10-day rule' for avoiding irradiation of unsuspected pregnancies. Stephenson enumerated some of the twenty-five new obligatory requirements in the code; he felt that reduction of patient dose was the area where improvements could be made.

The measures for increasing protection in radiotherapy were examined by T. J. Deeley (Velindre Hospital, Cardiff); again a more positive identification of hazards was evident in the new code. Leakage radiation from therapy equipment and diaphragm systems were unsatisfactory features leading to unnecessary patient irradiation.

That the section of the code dealing

with unsealed radioisotopes had required and received drastic revision was explained by N. G. Trott (Royal Marsden Hospital, Sutton), although he pointed out that the current revision had not fully kept abreast of recent changes in nuclear medicine technique. N. J. D. Smith (King's College Hospital, London) surveyed a large number of practices and revealed many with many unsatisfactory protection standards. Personnel monitoring had shown consistently high exposures to many dentists and ancillary staff, who were often unaware of the existence of the code of practice. It was reported that few dentists had taken advantage of a recent monitoring scheme launched by the National Radiation Protection Board. The need for a separate, explanatory supplement to the code for dentists was evident.

The afternoon session was devoted to problems in implementing the code, and the achievement of radiation safety in the hospital service. D. C. Holdsworth (United Hospital, Sheffield) thought that clinicians could make an effective contribution in reducing patient exposure, but they needed more information in order to weigh the risks of the radiation procedure against the hazards of omission. C. K. Warrick (Royal Infirmary, Newcastle upon Tyne) described his system for implementing the '10-day rule' in a survey 12 months ago; the rule was practised by only 20% of the departments sampled. Several hospitals had demonstrated that workable systems could be operated to reduce the number of unsuspected pregnancies irradiated.

The responsibilities of the Radiation Protection Adviser in achieving radiation safety were discussed by R. F. Farr (Queen Elizabeth Hospital, Birmingham), and N. Chesney, Farr's colleague, made a plea for a clear statement of the duties of the Radiological Safety Officer and, in many cases, appropriate training for these duties was required.

W. J. Meredith (Manchester Regional Hospital Board) spoke in the unusual guise of administrator, and pointed out that the code put a heavy responsibility on the controlling authority. Apart from the need to keep the numerous formal records required by the code, there was a continuing need to keep radiation safety committees alive and active.

R. D. Moore (Weston Park Hospital, Sheffield) described his attempts to evaluate the cost of protection measures. The annual cost in the United Kingdom of health physics services in the hospitals together with the costs of structural shielding amounted to some £580,000 per year. This resulted in a consequential saving of 140,000 man-rads, that is about £4 per man-rad saved.

Coulomb-nuclear effects

from our Nuclear Theory Correspondent

AN interesting example of interference between Coulomb and nuclear scattering amplitudes has recently been found in the inelastic scattering of heavy ions by nuclei. This interference can be understood classically in a qualitative way, and can also be calculated accurately using the distorted wave theory.

This interference is made possible by the particular character of the field between heavy ions, which allows incident particles with different classical impact parameters to be scattered through the same angle. To see how this comes about, consider the behaviour of the scattering angle as the impact parameter is steadily reduced.

For large impact parameters the projectile interacts only with the repulsive Coulomb field of the target, and is deflected through a small angle that steadily increases as the impact parameter is reduced. When the distance of closest approach becomes comparable to the sum of the radii of the projectile and target, the nuclear force begins to attract the projectile, thus reducing the scattering angle. At still smaller impact parameters the projectile is repelled by the centrifugal force, thus increasing the scattering angle again. The resulting variation of scattering angle is shown in Fig. 1 for 60 MeV ^{16}O ions on ^{58}Ni.

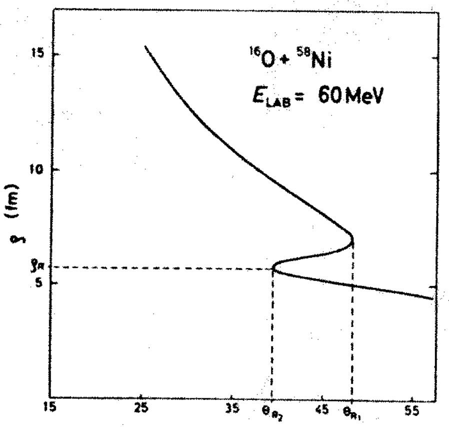

Fig. 1 Variation of the scattering angle θ with the impact parameter ρ for 60 MeV ^{16}O ions on ^{58}Ni (Malfliet, Landowne and Rostokin, *Phys. Lett.*, **44B**, 238; 1973).

In such cases there are three values of the impact parameter corresponding to each scattering angle between the critical scattering angles $\theta(R_1)$ and $\theta(R_2)$. The chief contribution to the scattering comes from angles around the critical angles, so a semi-classical theory can be made by adding the corresponding amplitudes, with appropriate phase and attenuation factors. The turning point corresponding to the larger impact parameter refers to the orbit further away from the nucleus where the projectile is moving mainly in the Coulomb field, whereas the other turning point refers to the orbit nearer the nucleus where the projectile is under the influence of both the Coulomb and nuclear fields. These amplitudes interfere, and because they vary with angle in a different way the interference is sometimes destructive and sometimes constructive.

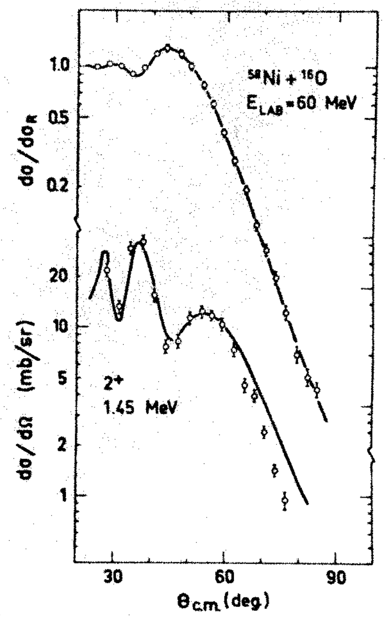

Fig. 2 Differential cross sections for the elastic and inelastic scattering of 60 MeV ^{16}O ions by ^{58}Ni showing the interference effects in the region of the critical angles. The curves are obtained using the distorted wave theory (Christensen, Chernov, Gross, Stokstad and Videbaek, *Nucl. Phys.*, **A207**, 433; 1973).

These effects appear in elastic scattering around the region of the critical angles, but are much more prominent in inelastic scattering. For small angles, the cross section for the excitation of the lowest $J^\pi = 2^+$ state of the target is almost entirely due to Coulomb excitation, while for large angles it is mostly due to nuclear excitation. In the intermediate regions around the critical angles the interference effects are strongly marked as shown in Fig. 2.

The whole process can be described quantitatively by the distorted wave theory, using the appropriate vibrational model for the 2^+ state. The results of such calculations are also given in Fig. 2 and show that the interference effects are accurately given by the theory. Detailed fitting in the interference region gives improved values of the parameters of the distorting potential and of the nuclear dynamical deformation parameter.

This interference phenomenon is a good example of the way interactions between heavy ions can be understood semi-classically and then analysed by the distorted wave theory to give additional information on nuclear structure.

Two Stage Red Sea Floor Spreading

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Evidence is presented which strongly suggests that most of the southern Red Sea is underlain by oceanic crust which evolved in two stages, the first from about 41 to 34 million years ago and the second from about 4 to 5 million years ago to the present day.

THE Red Sea consists of a main trough and a young axial trough which starts at about 24° N and widens towards the south[1]. Unfortunately, large amounts of sediment obscure the true structures especially in the southern half. It has been clear for some time that the axial trough is underlain by new oceanic crust but there are conflicting views on the nature of the crust underlying the main trough[1–14].

The scant amount of gravity data available in the 1950s was sufficient to show that the axial trough is underlain by a high density intrusive zone[15]. Further geophysical work confirmed the presence of this intrusive zone[1]. Large magnetic anomalies with amplitudes exceeding 1,000 nT were observed over the axial trough and seismic velocities of about 7 km s^{-1} were found at shallow depths of 2 to 3 km. The magnetic anomalies were later interpreted in terms of seafloor spreading and a half rate of 1 cm yr^{-1} was determined for latitude 16° N (ref. 3). It became clear that the Red Sea axial trough is being formed by seafloor spreading, and Arabia and Nubia have been drifting apart for the past 3 to 4 Myr.

The situation regarding the main trough and earlier history of the Red Sea has been much less clear. The fact that in many places the shelves are at or near sea level suggested in the 1950s that they are underlain by Precambrian shield rocks[15]. At that time the large thicknesses of sediments[9,16] were unknown. Further, seismic velocities of 5.9 km s^{-1} were found[1] suggesting the presence of at least some shield rocks. In the mid 1960s, the evidence (previously disputed) for about 100 km shear along the Dead Sea rift became very strong[17]. This 100 km displacement implies that there must be a much greater separation of the Red Sea than the width of the axial trough[2]. Seismic refraction experiments at 22 to 23° N with the objective of finding the extent of the oceanic crust[4,8] showed an average velocity of 6.63 ± 0.16 km s^{-1} at a mean depth of 4.6 km for the main trough, thus establishing the presence of oceanic crust and a minimum separation of Nubia and Arabia of 130 km. But some recent papers[12,14,18] still support the thesis that only the narrow axial trough is oceanic and the main trough and shelves are underlain by downfaulted shield rocks.

New evidence is given here which supports the existence of oceanic crust beneath the shelves of the southern Red Sea. In addition, it is now possible to be reasonably certain about the age of this crust.

New Magnetic Data

In 1966, the Gulf Oil Company flew a detailed total intensity magnetic survey at a height of 610 m over the western margin of the Red Sea between 14.5 and 16° N ; and in 1968, US Project Magnet flew eight lines (four at height 305 m and four at height 3,658 m) from shore to shore at 18° N.

The Gulf survey has excellent navigation control, Shoran being used. The original analogue records have recently been made available and have been digitised with the object of gaining information on the seafloor spreading history of the margins. The profiles were digitised at every Shoran location and additionally at every turning point so as to preserve the details of the records. This gave a digitisation interval of about 0.7 km. A ground magnetometer station was set up at Massawa and the records from here were used to correct for the daily variation. The IGRF 1965.0 gradient was removed from each profile.

For the Project Magnet profiles, Doppler navigation was used and hence these have less good positional control. The records were digitised in a similar way and the IGRF 1965.0 gradient removed.

Nine of the sixty Gulf profiles (G11, G16, G20, G25, G31, G35, G40, G44 and G50) and two of the eight Project

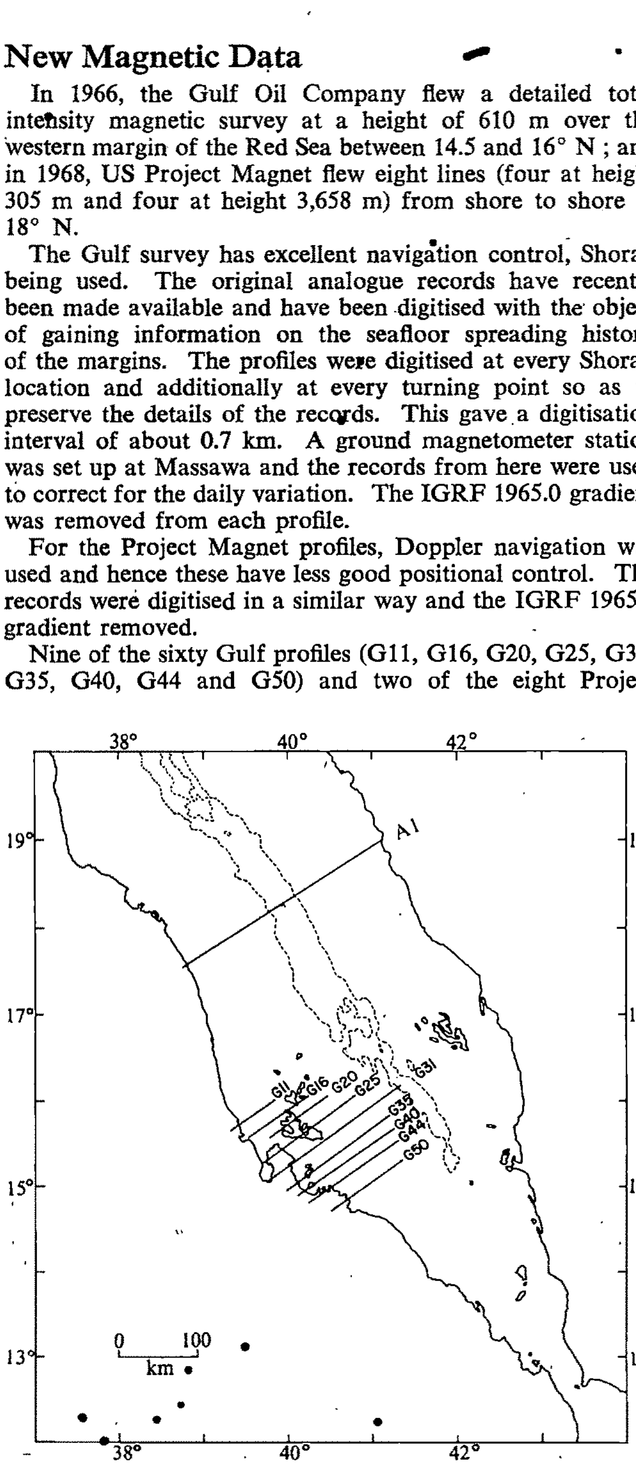

Fig. 1 Location of total intensity magnetic profiles. The dashed [illegible] are the 500 and 1,000 fathom contours taken from the 1 : 2 million bathymetric chart of Laughton[25].

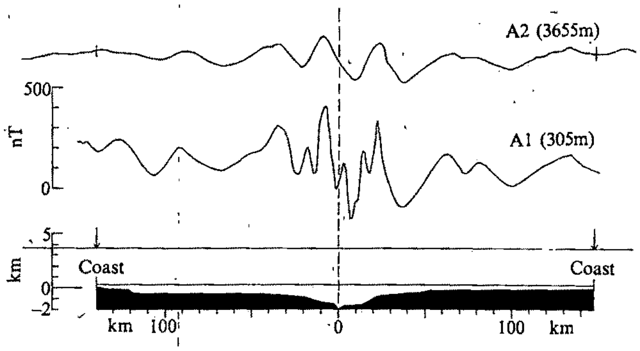

Fig. 2 Project Magnet profiles across the whole width of the Red Sea at 18° N showing the existence of magnetic anomalies on either side of the axial trough associated with symmetrical spreading (compare Fig. 5) and their relationship to the bathymetry.

Magnet profiles (A1 and A2) are used to illustrate the seafloor spreading history of the Red Sea. The locations of the profiles are shown in Fig. 1. A2 (height 3,655 m) has almost the same location as A1 (height 305 m).

Interpretation of Magnetic Anomalies

Figures 2 and 3 show two sets of examples of magnetic anomalies over the Red Sea. Figure 2 has two profiles flown at different heights from shore to shore (Project Magnet) showing the relationship of the anomalies to the bathymetry. Figure 3 shows a set of nine profiles over the western margin (Gulf Oil Co.). Taken together, these provide convincing evidence for the presence of oceanic crust beneath nearly all of the southern Red Sea.

First, the Project Magnet profiles (Fig. 2) show the familiar large anomalies over the axial trough and also the existence of anomalies over the main trough. It should be remembered that the computed anomalies for a normally magnetised body with the northwesterly strike of the Red Sea show a positive anomaly over its western edge and a negative over its eastern edge (a reversely magnetised body has a negative over the western edge and a positive over the eastern edge). When consideration is given to this, it is apparent that the anomalies are due to symmetrically emplaced bodies (Fig. 5) and the symmetry is impressive evidence that the anomalies are due to seafloor spreading. Figure 2 also shows a contrast in the amplitude of the anomalies over the axial trough and those over the margins suggesting two different phases of spreading. Further, the large anomalies are not exactly confined to the axial trough but extend somewhat further. Possible reasons for this are discussed by Girdler and Whitmarsh[19].

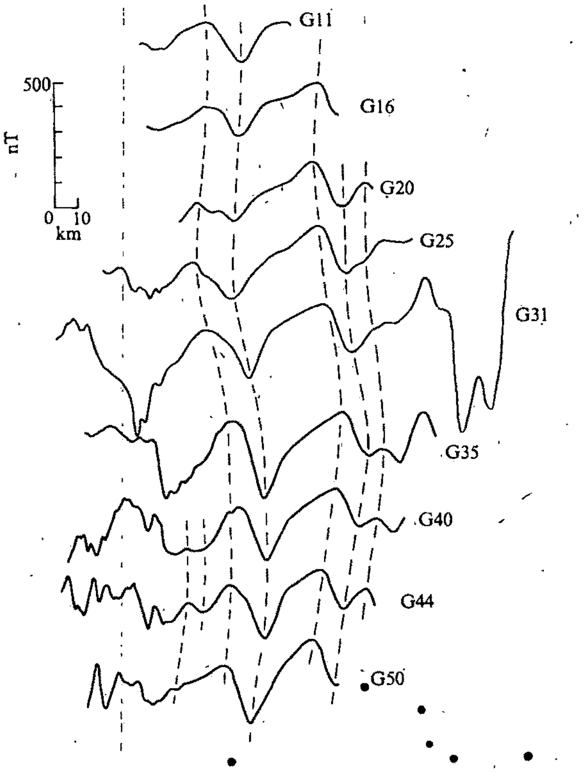

Fig. 3 Nine profiles from the Gulf magnetic survey illustrating the two types of anomalies, the smooth long wavelength well correlated anomalies over the Red Sea and the poorly correlated high frequency anomalies in the west.

Second, the Gulf profiles (Fig. 3) show that the anomalies on the western side of the axial trough are remarkably consistent and can be linearly correlated over a distance of at least 230 km. This would be very unusual for anomalies over shield rocks and provides additional evidence for their seafloor spreading origin.

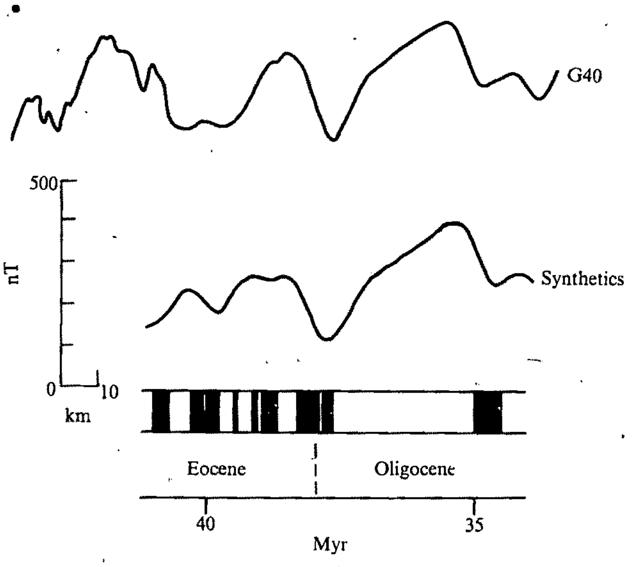

Fig. 4 Synthetic and observed profiles for Gulf profile G40. The synthetic profile is for a spreading rate of 1.4 cm yr^{-1} and strike N 120°.

Third, the Gulf profiles (G25, 31, 35, 40, 44 and 50) show a contrast between two types of anomalies—high frequency poorly correlated anomalies in the west and long wavelength well correlated smooth anomalies in the east over the Sea. Almost certainly the former are over Precambrian shield and the latter over seafloor.

Age of Red Sea Floor

Synthetic profiles were generated for the Red Sea using the reversal time scale of Heirtzler *et al.*[20]. At first, attempts were made to obtain an interpretation similar to that for the Gulf of Aden[21], that is, a continuous spreading history back to anomaly 5 (10 Myr). The correlations beyond 3 to 4 Myr were found to be very poor, as found by Vine[3]. The synthetic profiles were then extended back to the base of the Miocene (25 Myr) using various spreading rates and again good correlations could not be found. Finally, synthetic profiles were generated back to 70 Myr and searched for possible correlations with the observed anomalies.

A remarkably good correlation was found for the time interval 34 to 41 Myr. This is illustrated in Fig. 4 using Gulf profile G40. A spreading rate of 1.4 cm yr^{-1} over this time interval gives excellent correlations with the observed data. The long reversal interval (35.00 to 37.61 Myr) is necessary to obtain the characteristics of the Gulf profiles. Other possible candidates for this long reversal interval are even older (for example, 49.58 to 52.41 Myr) but for these the fits for the neighbouring anomalies are not nearly so good. It is therefore proposed that the oceanic crust to the sides of the axial trough is surprisingly old, being about 34 to 41 Myr (late Eocene to early Oligocene).

New Model for the Red Sea

A new geophysical model for the Red Sea is presented in Fig. 5. This utilises the shore to shore Project Magnet profile A1, the location of which is shown in Fig. 1. Various data such as thickness of sediments and seismic refraction profiles suggest that the old crust is at a depth of about 5 km and the new crust at about 2 km. The synthetic magnetics is compared with the observed. The fit of the anomalies for the older crust is not quite so good as for the Gulf data and this is considered to be partly due to the poorer navigation. The old phase is shown to end at about 34 Myr and the new phase to begin at just over 4 Myr. There is, of course, some uncertainty about these dates. It seems highly probable that the new phase of spreading may have commenced at the end of the Miocene (4.9 Myr ago[22]) when the whole sedimentary environment changed with the opening of the southernmost Red Sea to the Indian Ocean.

Support for the two stage model also comes from seismic reflection profiling[6,18]. The acoustic reflector (S) is now known from Deep Sea Drilling to be anhydrite and to represent the top of the Miocene[23]. This reflector is absent in the axial trough suggesting a second phase of spreading after the Miocene.

The model of Fig. 5 is in broad agreement with the heat flow seafloor spreading models of Sclater and Francheteau[24]. The axis of the Red Sea has very high heat flow consistent with the generation of new oceanic crust and the heat flow over the sides is slightly high[9] consistent with older oceanic crust.

Plate Tectonics

Figure 6 depicts a map of the Red Sea (Mercator projection) based on the 1:2 million bathymetric chart of Laughton[25] and showing the 500 and 1,000 fathom contours. The ages of the oceanic crust deduced from the magnetic profiles have been transferred to the map. For the Project Magnet data (18° N) the isochrons for the new phase of spreading are fairly easily correlated over all the profiles. It is less easy to correlate the anomalies for the old phase of spreading especially on the eastern side and the 35, 37 and 39 Myr isochrons are thus shown with dashed lines.

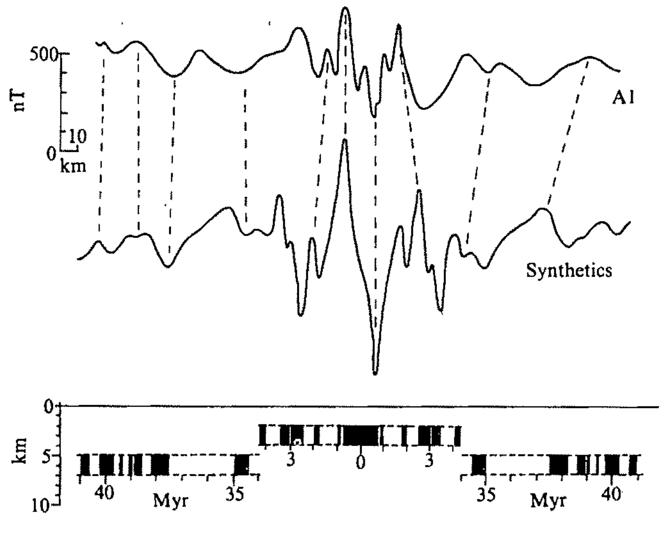

Fig. 5 A new geophysical model for the Red Sea (18° N) with two phases of seafloor spreading, the first (1.3 cm yr^{-1}) being in the late Eocene/early Oligocene and the second (0.9 cm yr^{-1}) being over the past few million years and still continuing.

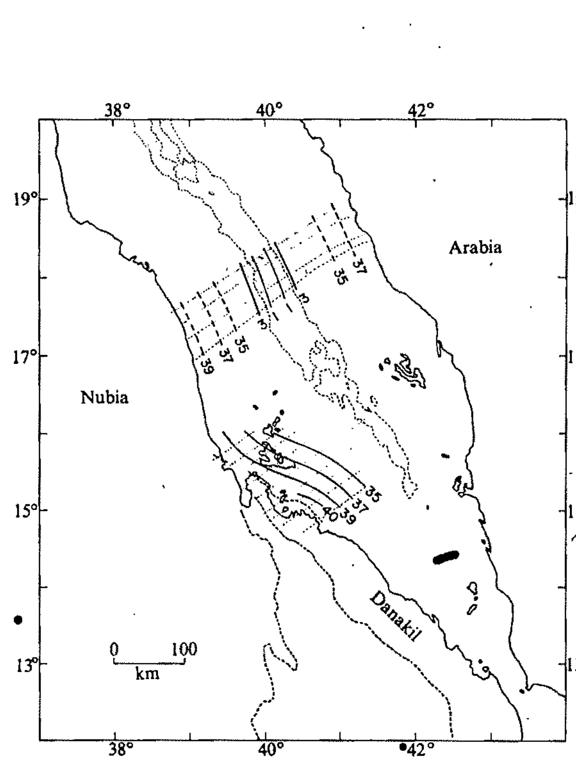

Fig. 6 Isochrons (Myr) for the ages of oceanic crust beneath the Red Sea. The dashed lines are less certain than the solid lines. The dotted lines are flight tracks.

From the Gulf magnetic profiles it is possible to locate the 35, 37 and 39 Myr isochrons with some confidence and to tentatively mark the oceanic/continental boundary of the Danakil plate. The isochrons bend southeastwards in this region. This is presumably because the Danakil plate was rotating counterclockwise with respect to the Arabian plate during this early phase of spreading. The anomalies can be seen to be closer towards the south (Fig. 3) especially for profiles G25 to G50. This is to be expected if Danakil was rotating with respect to Arabia during this time interval. To the north, away from Danakil, the anomalies are related to the counterclockwise rotation of Arabia with respect to Nubia.

Geological History of the Red Sea

In view of the evidence given here for an early phase of seafloor spreading, it is necessary to present a new summary of the geological history of the Red Sea and check that the model given in Figs 5 and 6 is consistent with the geological data. In particular, the geological summary[26] given by Girdler needs substantial revision (Fig. 7). The most important change is in the date of formation of the main Red Sea graben previously given as end of Oligocene/early Miocene, that is, about 26 Myr.

In the geological review given by Heybroek[27], it is reported that some kind of depression may have existed as early as Carboniferous times. The first major rift movements seem to have been in the lower/middle Eocene. Following these, a major phase of seafloor spreading occurred in the late Eocene and early Oligocene. This was followed by a long quiet period of about 30 Myr during which time huge thickn[illegible]f sediments, mainly evaporites, were deposited.

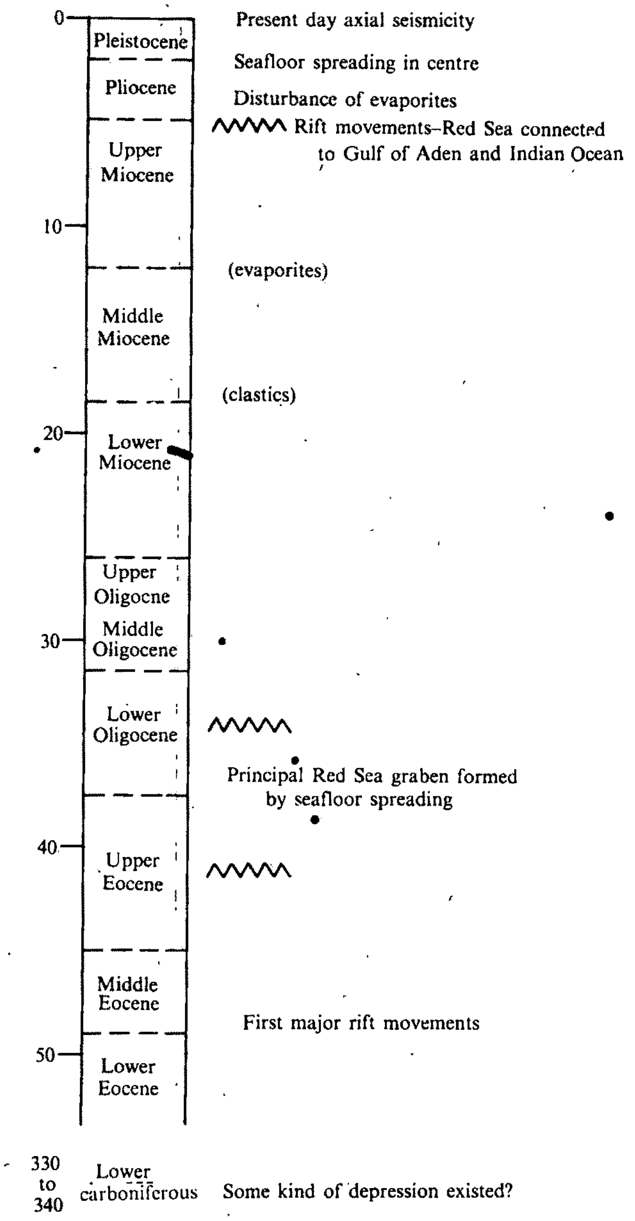
0
10
20
30
40
50
Pleistocene
Pliocene
Upper Miocene
Middle Miocene
Lower Miocene
Upper Oligocene
Middle Oligocene
Lower Oligocene
Upper Eocene
Middle Eocene
Lower Eocene
330 to 340
Lower carboniferous
Present day axial seismicity
Seafloor spreading in centre
Disturbance of evaporites
Rift movements–Red Sea connected to Gulf of Aden and Indian Ocean
(evaporites)
(clastics)
Principal Red Sea graben formed by seafloor spreading
First major rift movements
Some kind of depression existed?

Fig. 7 Geological history of the Red Sea.

From oil company data[12], the thickness of bedded evaporites is estimated to be as much as 5 km. Some K/Ar dates are available[12] from exploration wells in the south-west Red Sea (about latitude 16° N). These are 36.4±2 Myr and 36.6±2 Myr for the Amber well bottomhole core consisting of slightly metamorphosed very pyritic argillite and an average of 30 Myr for the bottomhole basalt core of well B1. These ages are no longer embarrassing, for it is now evident that the early stage of the Red Sea was in existence at least 40 Myr ago.

After the long 30 Myr quiet period of deposition, a new period of activity started about 5 Myr ago. The whole sedimentary environment suddenly changed at the end of the Miocene; nanno oozes were deposited instead of evaporites as the Red Sea became connected to the Gulf of Aden and Indian Ocean via the Straits of Bab-el-Mandeb. Seafloor spreading recommenced along the centre giving rise to large magnetic anomalies and very high heat flow. The axis of the Red Sea is seismically active and the process is still continuing[28]. The axial deep trough (Fig. 2) is due to the separation of old oceanic crust plus overlying sediments. The new oceanic crust beneath the axis reaches to within 2 to 3 km of sea level because of its higher temperature. The new phase of spreading caused considerable disturbance of the sediments with the creation of a very unstable situation as the crust and sediments parted and the axial trough evolved[19].

Some Implications

Finally, some implications concerning the possible existence of two stage Red Sea floor spreading are given.

The first concerns the Dead Sea rift. Freund *et al.*[29] find evidence for about 105 km shear, the whole of which can be dated as post-Cretaceous (about 65 Myr). This movement took place in two stages, the last 40 to 45 km being post-Miocene/early Pliocene. This correlates well with the second (recent) phase of spreading in the Red Sea. It has not yet been possible to date the remaining 60 km of shear[29] and it now seems likely that this was late Eocene/lower Oligocene corresponding to the first major phase of spreading in the Red Sea. It is to be hoped that Middle Eastern geologists will look for evidence to confirm this.

The second concerns the Gulf of Aden. Since little or no shear has been observed along the Ethiopian rift (between the Nubia and Somalia plates) it is to be expected that the history of the Gulf of Aden should be similar to that of the Red Sea. The interpretation of continuous seafloor spreading over the past 10 Myr in the Gulf of Aden[21] depends largely on the correct identification of anomaly 5 (not identified in the Red Sea). The magnetic profiles are therefore being re-examined to see if the two stage Red Sea spreading model also applies to the Gulf of Aden. Whitmarsh (personal communication) points out that as anomaly 5 is correlated into the north-west Indian Ocean, this is also in question. It may be that magnetic profiles over the oceans should be carefully re-examined for evidence of go-stop-go seafloor spreading.

Lastly, the Afar region (bordered by the Nubia, Somalia and Danakil plates) has been a popular location for a mantle hot spot. If hot spots provide the driving mechanism for plates, the evidence presented here suggests that this hot spot was active about 40 Myr ago, died for 30 Myr and relit about 5 Myr ago! This seems somewhat unlikely and it seems more likely that the intermittent plate motions of Arabia, Nubia, Danakil and Somalia are a consequence of more mantle wide convective motions.

We thank the US Naval Oceanographic Office and the Gulf Oil Co. for making available the original magnetometer records and for giving us permission to publish the profiles shown here. In particular, we acknowledge the help of Drs Henry Stockard and Patrick Taylor (USNOO) and Drs J. P. Huie (vice-president), A. C. Armstrong, M. D. Beltrandi and A. Pyre (Gulf). Stuart Hall helped with the digitisation of the Project Magnet records.

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Isolation of Bovine Thymin: a Polypeptide Hormone of the Thymus

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Two closely related polypeptides termed thymin I and II were isolated from bovine thymus by their effect in causing a delayed impairment of neuromuscular transmission *in vivo*. Purified thymin also induced bone marrow cells to develop the characteristics of intrathymic lymphocytes and it is probable that thymin is a lymphopoietic differentiating hormone of the thymus with secondary neuromuscular effects.

THE thymus is a compound organ consisting of an epithelial stroma derived from the third branchial arch and lymphocytes derived from stem cells originating in haemopoietic tissues (see ref. 1 for review). Lymphocytes differentiated within the thymus leave as mature thymus-derived cells (T cells) which circulate to the blood, lymph, spleen and lymph nodes. The induction of stem cell differentiation within the thymus is probably mediated by secretions of the epithelial cells of the thymus[2] but difficulties with bioassays have hindered the complete isolation and structural characterisation of these hormones[3–6].

A different approach to the isolation of thymic hormones was developed from experimental studies related to the human disease myasthenia gravis. Myasthenia gravis is characterised by a deficit in neuromuscular transmission and an associated thymic pathology has long been recognised in this disease. A study of clinical, pathological and immunological features of myasthenia gravis led to the hypothesis that thymic autoimmune disease is the lesion basic to this disease[7,8]. A laboratory animal model of experimental autoimmune thymitis was developed and these animals were shown to have an abnormality of neuromuscular transmission similar to that of myasthenia gravis by a number of criteria[9–14]. Experimental analysis of this laboratory model revealed that the effect on neuromuscular transmission was mediated by a substance released from the diseased thymus[9,13,14]. Neurophysiological studies in thymectomised and thymus grafted animals suggested that this substance was secreted physiologically[15]; and so the name thymin was proposed for this putative hormone.

The hypothesis of a neuromuscular blocking substance in the thymus is not novel; it was proposed in 1943 (ref. 16) in relation to the then current concepts of the curare-like nature of the myasthenic neuromuscular block, the known association of thymic pathology with the disease and the empirical discovery that thymectomy was an effective treatment for myasthenia gravis[17,18]. An unequivocal demonstration of such a neuromuscular blocking substance was not, however, obtained despite a large body of experimental work (reviewed in ref. 1). Although no consistent acute effects of thymus extracts could be demonstrated it was found that when thymus extracts were injected into animals impairment of neuromuscular transmission could be detected after a delay of several days[19]. This effect of a delayed production of impairment of neuromuscular transmission has served as a bioassay for monitoring the chemical fractionation of bovine thymus extracts.

I shall describe the isolation of two closely related polypeptides which produce, following a single injection of 4–32 ng per mouse, an impairment of neuromuscular transmission detectable by electromyography 1–5 d following injection. Of particular interest is the finding that these purified polypeptides also induce differentiation of bone marrow cells to T cells and hence seem to be thymic hormones which induce differentiation of the lymphopoietic stem cells which give rise to thymus-derived lymphocytes (T cells) (R. S. Basch and G. Goldstein, in preparation).

Assay Systems

Extracts were injected into test animals and neuromuscular transmission was studied after an interval of 1–5 d. Initially 100 g guinea pigs were injected intraperitoneally; neuromuscular transmission was studied *in vitro* with a preparation of the phrenic nerve and hemidiaphragm[19]. Subsequently 200 g rats were used; fractions were injected subcutaneously and neuromuscular transmission was assessed electromyographically[12]. Finally, mice were found to be an effective test system. Female Swiss-Webster mice weighing 25–35 g were injected intraperitoneally, groups of five to ten mice being used to test each fraction. The animals were anaesthetised with urethane and electromyography was performed as described previously[11]. The muscle action potentials were recorded in each mouse following supramaximal nerve

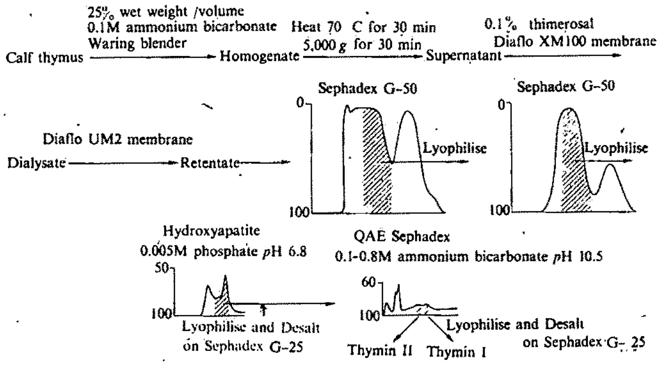

Fig. 1 Flow chart showing steps in the isolation of thymin I and II from bovine thymus. The chromatographic elution profiles are of transmittance at 254 nm and the shaded areas represent the fractions collected.

stimulation with ten impulses at a rate of 50 s^{-1}. Five sites were sampled in each mouse and for each group of mice the mean standard deviation and s.e.m. were calculated for the change in the height of the muscle action potential generated by the first and tenth impulses, the height of the tenth impulse being expressed as a percentage of the height of the first impulse.

Since this response in normal mice was found to vary somewhat with age and size, appropriate controls from the same group of mice were always tested. The statistical significance of differences found was assessed by Student's *t* test.

Purification procedures were also monitored by analytical polyacrylamide disc electrophoresis; 7% polyacrylamide gels were run at *p*H 8.9 and *p*H 4.3[20]. Methyl green was used as a dye marker in the acid gels run towards the cathode and bromophenol blue was used in the basic gels run towards the anode. Spacer gels were not used, the samples being loaded in electrode buffer made up with the dye marker in 25% sucrose. The gels were fixed in 12.5% trichloroacetic acid, stained with Coomassie blue and the relative mobilities (R_F) were calculated with respect to the dye markers.

The isolation methods are summarised as a flow chart in Fig. 1. Bovine thymus was obtained fresh on wet ice, dissected clean and stored at −20° C. Batches were homogenised 25% wet weight/volume in 0.1 M ammonium bicarbonate using a Waring blender. The extract was heated to 70° C for 30 min in a water bath, then centrifuged at 5,000*g* for 30 min. The supernatant was filtered through gauze and cotton and 0.1% thimerosal was added as a bacteriostatic agent since the subsequent steps were carried out at room temperature. Diaflo membranes (Amicon) and molecular sieve chromatography on Sephadex (Pharmacia) were used to obtain an enrichment on the basis of molecular size. The extract was processed through a Diaflo XM100A membrane in a TC10 apparatus. The dialysate was then concentrated over a Diaflo UM2 membrane, using a 402 cell pressurised to 55 pounds per square inch; 2 l was concentrated to 15 ml. This retentate was further fractionated by molecular exclusion chromatography on a 2.5×100 cm column of G-50 medium Sephadex (Pharmacia) in 0.1 M ammonium bicarbonate. The outflow was monitored with a Uvicord I monitor (LKB) which recorded ultraviolet transmittance at 254 nm. The fractions shown in Fig. 1 were lyophilised and rerun on the same column.

The lyophilised fractions from G-50 Sephadex were fractionated by adsorption chromatography on hydroxyapatite (Biorad). A 500 mg load was dissolved in 0.005 M sodium phosphate buffer, *p*H 6.8, and applied on a 2.5×30 cm column of hydroxyapatite in the same buffer. Active fractions were found retarded behind the void volume (Fig. 1); they were lyophilised and desalted on a G-25 Sephadex column. This material was fractionated further by ion exchange chromatography on a 1.5×30 cm column of QAE-Sephadex (Pharmacia) in 0.1 M ammonium bicarbonate brought to *p*H 10.5 with ammonium hydroxide. The column was developed with a linear concentration gradient to 0.8 M ammonium bicarbonate, also brought to *p*H 10.5. The two fractions shown in Fig. 1 were active in producing neuromuscular block. These were designated thymin II and thymin I (see below) and each was lyophilised and desalted on G-25 Sephadex in 0.1 M ammonium bicarbonate; the lyophilised void volume of these columns constituted the purified thymins. If these fractions were still impure on analysis by polyacrylamide disc electrophoresis the ion exchange chromatography step was repeated.

The yields varied somewhat from batch to batch; on average 1 kg of wet thymus yielded 500 mg of lyophilised powder after the Sephadex G-50 steps; this yielded 50 mg after hydroxyapatite adsorption chromatography and the final yield was approximately 1 mg of each thymin.

The electromyographic assay was adequate to detect peaks of activity during fractionation. Due to the eventual finding of two polypeptides, with slightly different thresholds of action and due to the wide variations in normal electromyographic responses which required large numbers of test animals and controls to assess variations statistically, it was, however, not possible to quantify enrichments and yields of activity for each step of the isolation.

Neuromuscular Action of Thymin

The electromyographic response of mice 5 d after various doses of thymin I or thymin II is summarised in Fig. 2. Bovine serum albumin (0.5 mg per ml 0.9% saline) was used as a protein stabiliser and controls were injected with the solvent alone.

In fifteen control mice tested at seventy-five muscle sites the tenth muscle action potential was 94±2% (mean±s.e.m.) of the first. For groups of mice (4–10) given varying doses of thymin I or II there was a statistically significant ($P<0.001$) augmentation of the degree of decrement at most doses above a threshold which was 4 ng per mouse for thymin I and 32 ng per mouse for thymin II. The degree of neuromuscular impairment did not increase with increasing dose above the threshold and the mean response for all mice given suprathreshold amounts of thymin was 84% for thymin I and 82% for thymin II (Fig. 2).

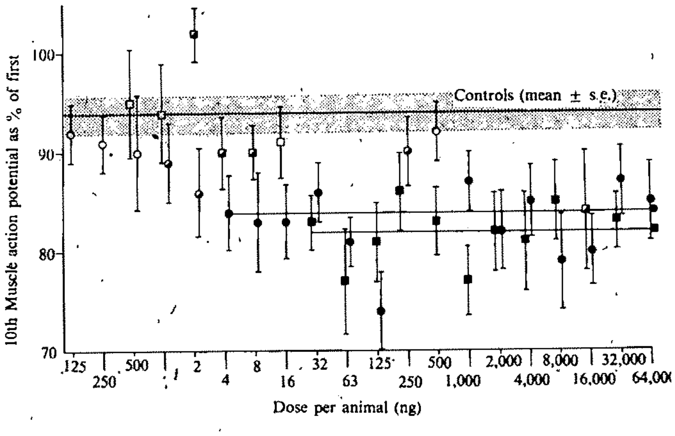

Fig. 2 Chart showing the electromyographic response of mice to supramaximal nerve stimulation at a rate of 50 impulses s^{-1}. The mean values and s.e.m. (vertical bars) are shown for groups of 4–10 mice given various doses of thymin I (○) and thymin II (□). Statistical significances of differences from the controls were calculated by Student's *t* test and are shown by solid symbols ($P<0.001$), half solid symbols ($0.05>P>0.001$) and open symbols ($P>0.05$).

Mice were given 100 ng of thymin I or solvent and five mice from each group were tested at various times. A statistically significant ($P<0.001$) effect on neuromuscular transmission was first detected 18 h after injection, became maximal by 24 h and persisted through 5 d (Fig. 3).

Relationship of Thymin I and Thymin II

During the development of the isolation procedures for thymin, the purity of fractions was assessed by polyacrylamide disc electrophoresis at *p*H 8.9. Neuromuscular blocking activity was found associated with material which stained with Coomassie blue, and had an R_F of 0.30 with respect to bromophenol blue (Fig. 4). At *p*H 8.9 thymin I migrated only slightly faster than thymin II. At *p*H 4.3, however, this material resolved into two distinct bands both of which produced neuromuscular impairment (see above). The faster one was designated thymin I (R_F 0.63 with respect to methyl green) and the slower one thymin II (R_F 0.57) (Fig. 4). The mobilities of these purified polypeptides remain consistent and do not interconvert.

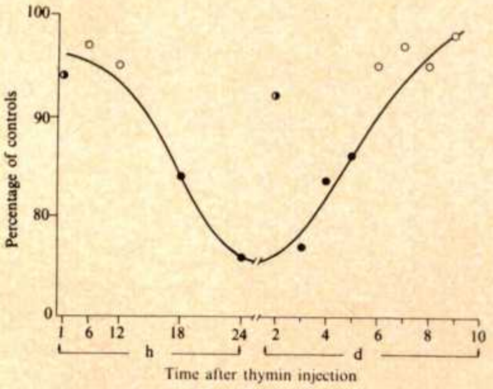

Fig. 3 Electromyographic response of mice at various times after a single injection of 100 ng thymin I expressed as a percentage of the response of mice injected with solvent alone. With supramaximal nerve stimulation at 50 impulses s^{-1} the height of the tenth muscle action potential was expressed as a percentage of the first. This change was calculated for five points in each of five to ten mice from each group. For each time point the change in thymin-treated mice was expressed as a percentage of the change in solvent-treated control mice and the statistical significance of the difference was assessed by Student's *t* test; solid circles: $P<0.001$; half solid circles: $0.05>P>0.001$; open circles: $P>0.05$.

Thymin I and II are polypeptides with molecular weights of approximately 7,000, based on their characteristics in molecular sieve chromatography and their amino acid compositions. Preparations of thymin I and II were pure by the criterion of polyacrylamide disc electrophoresis. With 200 μg of thymin I or II per gel there was a single major band stained with Coomassie blue in gels run at *p*H 8.9 or *p*H 4.3. Analysis of the terminal amino acids provided further evidence for purity. No residue could be identified at the N terminus of thymin I or II which was therefore considered blocked. Only lysine was identified at the C terminus of both thymin I and II (D. S. Schlesinger, G. Goldstein and H. D. Niall, in preparation).

Thus thymin I and thymin II seem to be very similar. They were closely associated during the various fractionation procedures and it was difficult to establish chromatographic conditions that would resolve them. The presence of at least some areas of common primary amino acid sequence was suggested by two sets of observations. Thymin I and thymin II were digested with trypsin and the tryptic peptides were mapped in two dimensions by chromatography and high voltage electrophoresis. Several peptides with similar positions were found in thymin I and thymin II, suggesting common sequences. Rabbit antisera raised against either thymin I or thymin II reacted with both and in two dimensional immunodiffusion on micro-Ouchterlony plates thymin I and thymin II produced a line of identity (Fig. 5). This again provided evidence that thymin I and thymin II had a common tertiary configuration being recognised by the antibodies and so probably had some common primary sequence.

Yet there is a consistent biochemical difference between thymin I and II as detected by ion exchange chromatography at *p*H 10.5 and polyacrylamide disc electrophoresis at *p*H 4.3. Although the evidence summarised above suggests that thymin I and II share extensive primary sequence it is still uncertain whether one represents a chemically modified form of the other or whether they are two distinct polypeptides

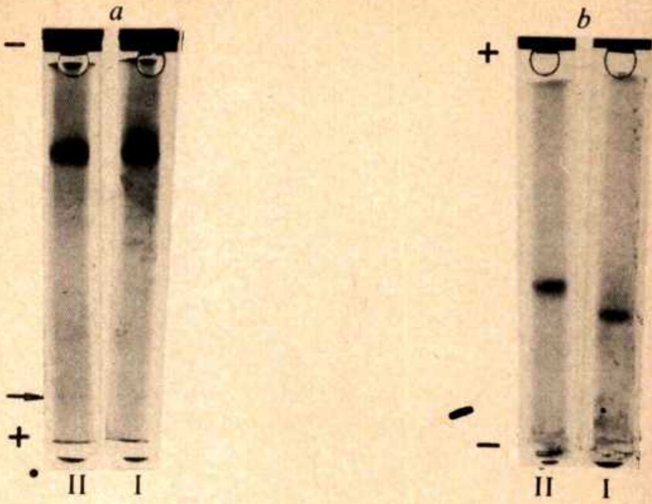

Fig. 4 Polyacrylamide gels from disc electrophoresis of 200 μg samples of thymin I and thymin II, *a* at *p*H 8.9 and *b* at *p*H 4.3. The gels were fixed with trichloroacetic acid and stained with Coomassie blue. One major band is present in each gel. Arrow indicates dye marker.

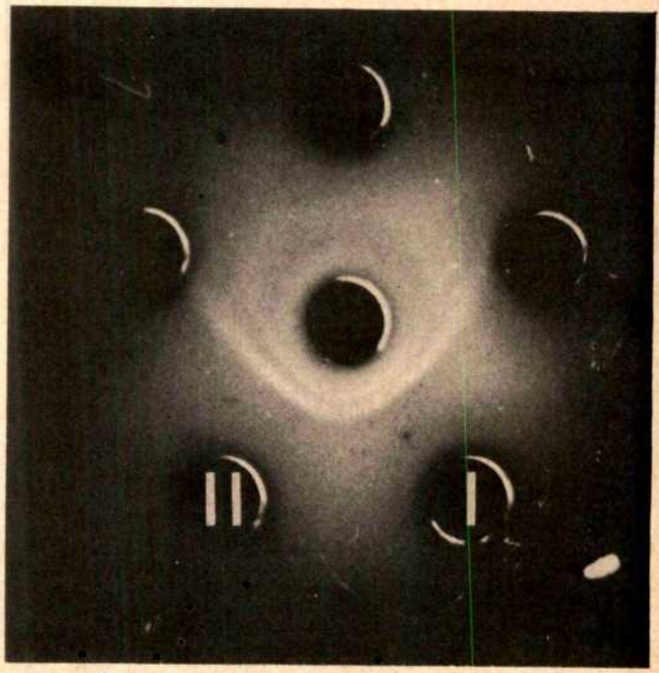

Fig. 5 Micro-Ouchterlony immunodiffusion with antiserum to thymin I in the centre well, thymin II (1 mg ml^{-1}) in the lower left well and thymin I (1 mg ml^{-1}) in the lower right well. After 24 h diffusion the plate was fixed in trichloroacetic acid and stained with Coomassie blue for photography.

with close sequence homology based on a common ancestral gene.

Biological Significance of Thymins

An interpretation of the findings in myasthenia gravis as suggestive of autoimmune thymitis[7,8] led to the development of a model of the disease in laboratory animals, experimental autoimmune thymitis[9-14]. Experimental analysis of this model implicated a substance released from the diseased thymus in the pathogenesis of the neuromuscular lesion[9,13,14] and experiments on thymectomised and thymus-grafted animals indicated that this substance was also secreted by the normal thymus[15]. This thymic substance affecting neuromuscular transmission was termed thymin.

The prime function of the thymus is the differentiation of lymphopoietic stem cells originating in the haemopoietic tissues to mature thymus-derived cells (T cells) capable of involvement in the immune response of the body[2,6]. Since this differentiating function is probably mediated by a hormone secreted by the epithelial cells of the thymus the finding of a hormone affecting neuromuscular transmission raises two possibilities. The thymus may secrete two separate hormone systems, one differentiating lymphopoietic stem cells, the other affecting neuromuscular transmission. Alternatively, by virtue of some similarity in the receptors of lymphopoietic stem cells and the neuromuscular synapse, the one hormone may affect both.

Purified thymin I, in subnanogram concentrations, caused a detectable neuromuscular lesion and also induced the differentiation of bone marrow cells *in vitro* in that they acquired antigens characteristic of intrathymic T cells (R. S. Basch and G. Goldstein, manuscript in preparation). Thus these findings suggest that thymin(s) are primarily hormone(s) of the thymus, inducing differentiation of lymphopoietic stem cells and that the effect on neuromuscular transmission is secondary and may be related to the presence of some receptor in the synaptic apparatus which is similar to that of the stem cells. Cholinergic receptors are present on both stem cells[21] and mature lymphocytes[22], and it is possible that the receptors of stem cells which induce differentiation to T cells may have evolved from such cholinergic receptors.

By this reasoning the neuromuscular effect of thymin is a chance one, rather than a primary physiological regulating function of the body. Nevertheless, the mode of action of thymin in producing neuromuscular block is of great interest. Although thymin results in a postsynaptic block in neuromuscular transmission[12] the evidence is that it does not combine directly with the classical acetylcholine receptor since it is inactive *in vitro*[19], causing neither acute blocking nor depolarisation, and it has an 18 h latent period *in vivo*, the effect then persisting for several days. These findings would suggest that thymin is affecting a regulatory mechanism in the neuromuscular synapse and thymin may be a valuable tool for probing such a mechanism of chronic modulation of synaptic conductivity.

This work was supported by the Irvington House Institute and grants from the National Institute of Neurological Diseases and Stroke of the US Public Health Service, the Muscular Dystrophy Associations of America and the Sackler Research Fund. Ronald King and Miriam Siegelman provided technical assistance. I am grateful for the advice and collaboration of Drs H. N. Niall, D. S. Schlesinger, R. S. Basch and M. E. Lamm.

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Structure of Yeast Phosphoglycerate Kinase

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A 3.5 Å resolution electron density map of yeast phosphoglycerate kinase has been calculated from which a skeletal model of the enzyme's single polypeptide chain has been constructed. The position of the nucleotide substrate ADP when bound to the enzyme has been defined.

We recently reported the structure of yeast phosphoglycerate kinase (PGK) as seen at 5 Å resolution[1]. This work and that of Blake *et al.*[2], who have carried out similar crystallographic studies with horse muscle phosphoglycerate kinase, have shown that this monomeric enzyme is composed of two lobes connected by a waist region. A 3.5 Å resolution electron density map of the yeast enzyme has now been calculated, from which it is possible to follow the course of the polypeptide chain starting in one lobe and passing through the central waist to form the structure of the second lobe before returning to complete the structure of the first lobe.

The reaction catalysed by PGK is the first of the two ATP-forming reactions of the glycolytic pathway. By soaking crystals of the enzyme in solutions containing nucleotide substrates we have also located the position of a unique Mg-ADP binding site near the surface of one of the two lobes.

Structure Determination

Two single-site heavy atom derivatives of PGK were obtained by soaking crystals with mercuric acetate or sodium aurichloride respectively, as previously described[1]. X-ray intensity data to 3.5 Å resolution were collected from the native protein using a computer-controlled four-circle diffractometer. These 7,820 measurements were divided into sets on the basis of intensity, each set containing approximately one-tenth of the X-ray reflections. Data for each of the two derivatives were collected for the six sets of highest intensity, totalling 3,752 independent Bijvoet pairs. Scaling of derivative to native data and the refinement of heavy atom parameters were carried out separately for each set of reflections. Anomalous scattering data were included in refinement and phase calculation procedures. The mean figure of merit[3] for all measured reflections was 0.76. The electron density distribution of the native enzyme, calculated using centroid phases[4], was plotted on Cobex sheets, in sections of constant y on a scale of $10^8:1$. A molecular model was built using Nicholson model components (Labquip, Reading, England), which were fitted to the electron density using an optical comparator[5]. In the absence of sequence information, each amino acid residue was inserted as L-alanine.

Interpretation of Electron Density

For much of its length, the course of the polypeptide chain could be followed without difficulty, and density corresponding to the larger amino acid side chains was clearly visible. Helical regions of the polypeptide chain were also immediately apparent, as is shown in Fig. 1. Since the correct 'hand' of the protein had been determined using the anomalous scattering data[1], the polarity of the chain could be defined by noting the orientation of the side chains in helices and in some runs of extended chain. As expected at this resolution, the main chain carbonyl oxygens were not resolved from the rest of the main chain, so that the orientation of the peptide bonds could not be determined directly. The planar peptide groups were therefore adjusted to give the best fit to the main chain density, and to locate β-carbon atoms at side chain density where this was clearly indicated. At bends in the chain, it was sometimes impossible, in the absence of sequence information, to tell how much of the density was due to main chain and how much to side chain atoms, so that an amino acid may have been omitted or inserted in such places. Also, the close approach of several runs of chain makes it difficult to determine, by immediate inspection, the correct connection between strands entering and leaving two small inadequately resolved sections of the molecule. The correct interpretation must produce for the molecule as a whole a single, continuous polypeptide chain with each element of the secondary structure having the correct polarity. This criterion was used to resolve any ambiguity in difficult regions of the map.

Knots in the polypeptide chain would not be expected to arise during spontaneous folding of the protein, but might easily be produced in a model by misinterpretation of the electron density map. In this case it is possible to trace the entire course of the chain starting from the amino terminus without having to pass the growing carboxyl end through any loop of chain already produced. It must be emphasised, however, that, apart from any possible error in the X-ray measurements, the absence of sequence information and the possible existence of flexible sections of polypeptide chain make certainty of interpretation unattainable at this stage in our investigation. We nevertheless believe that the structural information now available may be of considerable use in providing a conceptual framework for those interested in the structure and mechanism of kinases.

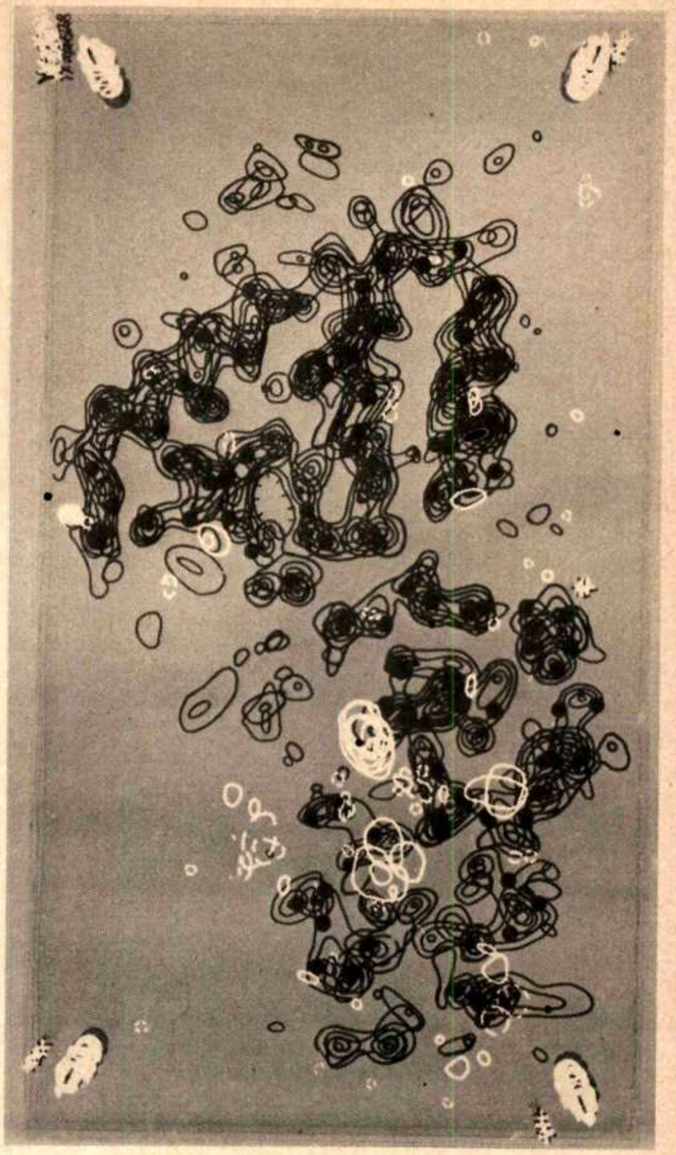

Fig. 1 Sections through the electron density map of PGK (black contours) with the difference electron density map of PGK+(Mn-ADP) minus PGK superimposed. Lowest black contour is at 0.35 e Å^{-3} followed at intervals of 0.15 e Å^{-3}. Broken white contours are negative, full ones positive. White contours are drawn at intervals of 0.06 e Å^{-3} with the zero contour omitted. The positions of α carbon atoms are marked with black disks. The axis of helix B2 lies in the plane of the sections and can be seen running from the top centre to middle left hand side of the map. Lobe B is above lobe A as illustrated. The peak assumed to be associated with the metal-phosphate end of the ADP molecule (peak 1) is the peak contoured in white nearest the centre of the map. The difference peak immediately below peak 1 is thought to represent the adenine portion of the ADP molecule. Other difference electron density peaks do not exceed one contour level on any one Fourier section.

The PGK Molecule

The separation of the monomeric PGK molecule into two lobes connected by a central waist region[1,2] is now confirmed. The polypeptide chain crosses twice between the two lobes, once in each direction. This means that both chain termini must occur in the same lobe, lobe A. The other lobe, lobe B, contains the yeast enzyme's single sulphydryl group, as judged from the positions of our two heavy atom sites[1].

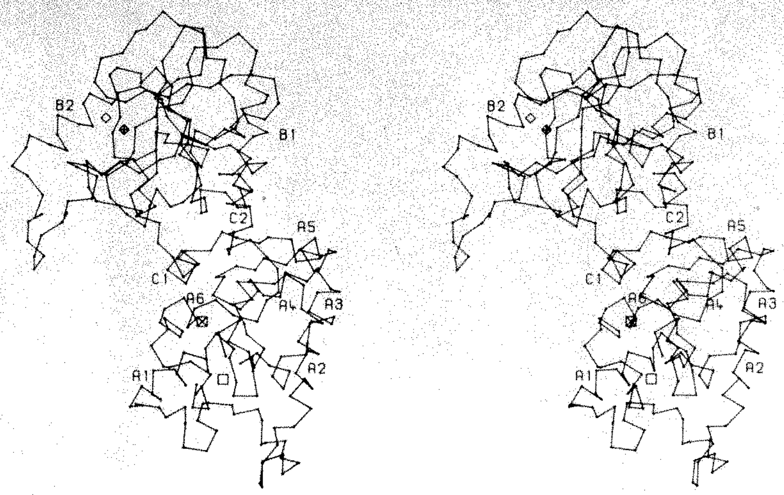

Fig. 2 Stereoscopic drawing of the PGK molecule viewed in the same orientation as the electron density map shown in Fig. 1. The positions of the α carbon atoms are marked so as to help the viewer follow the course of the main chain. The helical segments are labelled A1–A6, B1–B2, and C1–C2, to indicate the lobe or central waist region and numerical order of occurrence along the chain from the amino end. The positions of the two heavy atom sites are marked by open (Au) and crossed (Hg) diamonds respectively. The positions of the two significant difference Fourier peaks (see text) are shown with open (peak 2) and crossed (peak 1) squares respectively.

Of the total of 357 residues built into our model, 157 are in lobe A and 181 in lobe B, the remaining nineteen residues forming the connecting region between the two lobes.

Each of the two main chain connections between the lobes includes a short helical segment, designated C1 and C2. Inspection of the electron density map also shows five contact areas involving amino acid side chains which could be important in maintaining the two lobes in their relative orientations. On one side of the molecule, a residue near the carboxyterminal end of helix C1 (see stereo diagram) is in contact with a residue in helix A6. The amino terminal residue of helix C1 is in contact with another, non-helix, residue in lobe A, and the preceding residue is in contact with a non-helix residue in lobe B. At the other side of the molecule, two residues in helix C2, separated by a single amino acid, are in contact with residues in lobe A and lobe B respectively.

The molecule as a whole contains ten helices, six in lobe A, two in lobe B, and the two already referred to as forming part of the inter-lobal connection. Altogether some eighty-six residues, or one-quarter of the molecule, are involved in these helical segments, which vary in length from six to thirteen residues.

Although the existence of interchain hydrogen bonds cannot be established directly at this resolution, there are regions in each lobe where sections of extended polypeptide chain run sufficiently close together to suggest that a β pleated sheet is formed. These β pleated sheet regions tend to form the core of each lobe, and seem to be protected from the solvent by the helices, which all lie approximately tangential to, and form part of, the surface of the molecule. In lobe A, three strands running parallel in direction and sense form a plane between helices C2 and A3. Adjacent to this plane, the amino terminal part of the chain folds to form a compact double bend whose three short constituent sections lie close together in a plane between helices A6 and A2. These two possible regions of β pleated sheet also have a short common boundary whose adjacent chains run in opposite directions. In the other lobe, a twisted sheet is formed, between helices B1 and B2, by five chains, the middle one of which runs from the carboxy terminal end of helix B1 (the top of helix B1 as shown in the stereo diagram).

At two places in the molecule, for a distance of one amino acid residue, the main chain seems to pass through negligible electron density. In one further place there is a similar gap at the tip of a loop protruding into the external solvent. Although we have bridged this gap with a single amino acid, the conformation and position of this region would be consistent with the presence of a larger loop. Such a large flexible loop would not be revealed using X-ray diffraction techniques, and might help to explain the discrepancy between the 357 residues built into our model of the enzyme and the 420 residues calculated to be present using molecular weights determined by other physical methods[6,7]. We believe, however, that this discrepancy is such as to suggest that solution studies have led to overestimation of the molecular weight of PGK.

Structure and Reactivity

The heavy metal ligands used in the structure determination bind to the enzyme at two points 6 Å apart between two chains near the surface of lobe B. Although it is not possible at this stage in the investigation to indicate which of these two chains contains the molecule's single cysteine residue, it is quite clear that this residue is some 30 Å from the catalytically active site of the enzyme, as is shown in the stereo diagram. This is consistent with the finding that the cysteine residue is not essential for enzymic activity[7,8].

Substrate binding studies with yeast PGK are still at a preliminary stage. Changes in X-ray diffraction intensity have been observed for crystals soaked in the presence of adenosine[9], and of ADP with and without Mg^{2+} or Mn^{2+}, but not with 3-phosphoglycerate. Complete sets of 5 Å resolution X-ray diffraction data have been collected for the promising derivatives, and various combinations of difference electron density maps have been calculated. These maps show two consistent features, both positive, as is illustrated in Fig. 1, where the peaks associated with the PGK+(Mn–ADP)−PGK difference map are superimposed on equivalent sections of the normal electron density map. These peaks, designated peak 1 and peak 2, are shown in the stereo diagram with crossed and open squares respectively. Both peaks normally seem to be at least three times the general error level and are easily the strongest features in the calculations. In difference electron density maps between PGK+(Mg–ADP or Mn–ADP) and PGK alone, peak 1 is about 50% stronger than peak 2, whilst in the PGK+(Mn–ADP)−PGK+(Mg–ADP) difference map peak 1 is three times as strong as peak 2. These results suggest that peak 1 represents the phosphate-metal end of the ADP molecule whilst peak 2 represents the adenine portion. The two peaks are positioned in space in such a way that an ADP molecule can be built into the observed density which is fully consistent with this interpretation; the nucleotide substrate making very reasonable contacts with the protein without the need for any obvious change in conformation. Presumably the sugar moiety is not seen in the difference maps because it displaces some other molecule or group of molecules when the enzyme, in the crystal, binds ADP.

The picture of the PGK molecule which is now emerging thus focuses our attention on one region in particular. This is the 20 Å-deep wedge-shaped space between the two lobes, with helix C1 at its apex, which is on the same side of the molecule as the single sulphydryl group. This area contains, besides several points of inter-lobal contact, the nucleotide binding site. One may therefore expect that the residues involved in the transfer of the bioenergetically important phosphate group will be found in this region. We also note that the main structural features of the nucleotide binding lobe are similar to those which have been shown to surround the NAD binding site of several dehydrogenases[10-12]. A detailed comparison of the kinase and dehydrogenase binding sites must await extension of the present investigation. From the evidence now available, however, it seems probable that evolution has produced a general method for binding nucleotide substrates, and that the architecture of this binding site has been conserved for enzymes requiring such a function, including kinases.

Results comparable to those described here for yeast PGK have been obtained by Dr Blake and his colleagues at Oxford for the horse muscle enzyme. We thank the Oxford group for showing us the results of their work before publication. We also thank the Science Research Council for supporting our laboratory and for the award of a research studentship to one of us (T. N. B.).

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Production of Superheavy Nuclei by Multiple Capture of Neutrons

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A new calculation of fission barriers and neutron separation energies for heavy neutron-rich nuclei indicates that it is unlikely that superheavy nuclei can be produced either in the astrophysical r process or in man-made nuclear explosions.

PREDICTION of the possible existence of superheavy nuclei has led to attempts to produce them in the laboratory and to searches for them in nature. Calculations of the nuclear properties of such nuclei[1-4] have predicted half-lives as long as 10^9 yr. Thus, if these nuclei are produced in astrophysical environments, they should be detectable on the Earth and in cosmic rays impinging on the Earth. So far efforts to produce them in the laboratory or to find them in nature have failed.

There has been much interest in whether superheavy nuclei can be produced in the astrophysical r process, which is known to produce many of the naturally occurring neutron-rich nuclei between germanium and bismuth and all of the naturally occurring nuclei heavier than bismuth. The r process, occurring during some catastrophic supernova event, is the capture of neutrons on a time scale much faster than beta decay. Different studies have led to conflicting conclusions[5-15] as to whether superheavy nuclei can be produced by the r process. The important question is where neutron-induced fission terminates the r-process path in the region of heavy neutron-rich nuclei. This termination depends on how rapidly the effective surface tension of a nucleus decreases with increasing neutron excess and on the extra stability against fission gained from single particle effects near the neutron closed shell at $N=184$. The treatment of these two effects depends first on an extrapolation of the surface tension of nuclei along the valley of beta stability to nuclei far to the neutron-rich side of the valley of beta stability. Second, it depends on an extrapolation of the additional binding at known closed shells to the predicted closed shell at $N=184$. The various treatments of these two effects have resulted in the conflicting conclusions.

In this paper, we re-examine the question of the formation of superheavy nuclei by the r process, as well as their possible production in thermonuclear explosions, either of the conventional type or of the multistep type proposed by Meldner[16]. Figure 1 illustrates the path each of these processes would follow in the neutron-rich region in their approach to the island of superheavy nuclei.

Nuclear Potential Energy of Deformation

Since neutron-induced fission plays the critical role in terminating these processes, we calculate the fission barriers and neutron separation energies for a broad region of heavy neutron-rich nuclei, outlined by the dashed lines in Fig. 1. We use an improved version of the macroscopic–microscopic method, in which the smooth trends of the nuclear potential energy of deformation are calculated from a macroscopic model and the local fluctuations from a microscopic model. This method is described completely in recent review articles[17-21].

We pay special attention to the effective nuclear surface tension by using the droplet model of Myers and Swiatecki[22] for the macroscopic energy. This model takes into account higher order terms in $A^{-1/3}$ and in $[(N-Z)/A]^2$ than are retained in the usual liquid drop model, where A is the mass number and $(N-Z)/A$ is the relative neutron excess. To calculate the microscopic corrections to the energy we use a

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realistic diffuse surface single particle potential[3,4]. The single particle potential parameters are obtained from statistical calculations[23] and from adjustments to reproduce experimental single-particle levels in heavy deformed nuclei[21]. The approach that we follow here reproduces experimental fission barriers in the actinide region and nuclear ground state masses[21] to within an accuracy of about 1 MeV and is consistent with information from conventional nuclear explosions in the neutron-rich region[24].

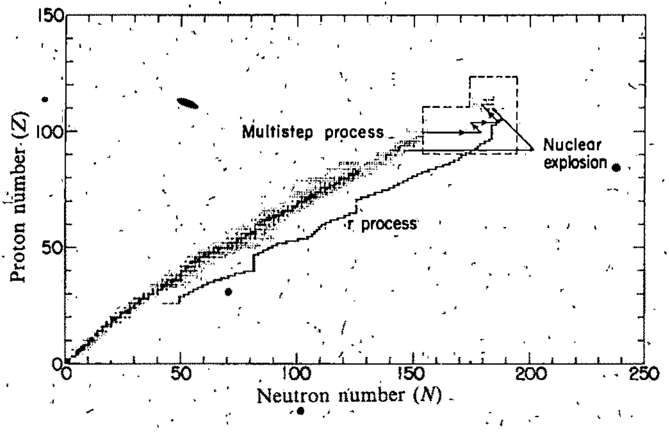

Fig. 1 Plot of known and predicted nuclei as a function of the neutron number N and proton number Z. The heaviest points represent stable nuclei, the medium size points nuclei with halflives greater than one year, and the lightest points nuclei with halflives less than one year. Nuclei with predicted halflives greater than about 5 min are included in the island of superheavy nuclei. The dashed lines outline the region of nuclei where we calculated fission barriers and nuclear ground state masses. Three possible ways to reach the island of superheavy nuclei by the multiple capture of neutrons are illustrated.

The neutron separation energies are calculated from ground state masses that are determined by minimising the potential energy with respect to the nuclear quadrupole moment Q_2 and hexadecapole moment Q_4. The fission barriers are calculated for a prescribed sequence of nuclear shapes relevant to the fission process. These details are discussed elsewhere.

Effective Surface Tension and Neutron Excess

In the liquid drop model for the macroscopic energy, the surface energy of a spherical nucleus is given by

$$E_s^{(0)} = a_s[1-\kappa((N-Z)/A)^2]A^{2/3} \quad (1)$$

where a_s is the surface energy constant and κ is the surface asymmetry constant. For a large value of κ, the effective surface tension decreases rapidly with increasing neutron excess, which results in decreased barrier heights for heavy neutron-rich nuclei. In the liquid drop model of Myers and Swiatecki[25] the surface asymmetry constant was assumed equal to the volume asymmetry constant, or to the value $\kappa = 1.7826$.

In the droplet model the shape dependence of the potential energy is more complicated than in the liquid drop model, and the constant κ no longer enters. But by neglecting the relatively small influence of some of the new shape-dependent energies, the more complicated dependence of the droplet model on the surface energy can be described[26] approximately in terms of an effective value of κ. Unlike in the liquid drop model, this effective value of κ depends on the mass number and the nuclear shape.

With the droplet model parameters of Myers and Swiatecki[22], we obtain $\kappa_{eff} = 2.8$ for the neutron-rich nucleus ^{280}Pu. Thus the effective surface tension decreases much more rapidly with neutron excess than in that liquid drop model. A recent determination of the constants of a truncated droplet model that reproduces both experimental ground state masses and fission barrier heights to within an accuracy of 1 MeV yields a value for ^{280}Pu of $\kappa_{eff} \approx 3.5$ (W. M. H. and P. A. Seeger, in preparation).

Fission-Barrier Heights

Figure 2 is a contour plot of the calculated fission-barrier height for even nuclei. We include a zero-point energy of 0.5 MeV for motion in the fission direction. Notice the large barriers ($\approx$10 MeV high) for nuclei in the superheavy region ($N \approx 184$, $Z \approx 114$). The barriers for these superheavy nuclei are similar to those that others calculate with a diffuse surface potential[3,4,18,20].

The barrier heights for nuclei in the region $176 \lesssim N \lesssim 192$ are largely the result of single particle effects because the macroscopic energy contribution has almost disappeared due to the large effective surface-asymmetry constant. Notice the pronounced enhancement of barrier heights for nuclei with neutron numbers near $N = 184$. In the broad region of nuclei $162 \lesssim N \lesssim 178$, $92 \lesssim Z \lesssim 106$ with relatively low fission barriers the capture of a neutron by these nuclei would lead to disastrous neutron-induced fission.

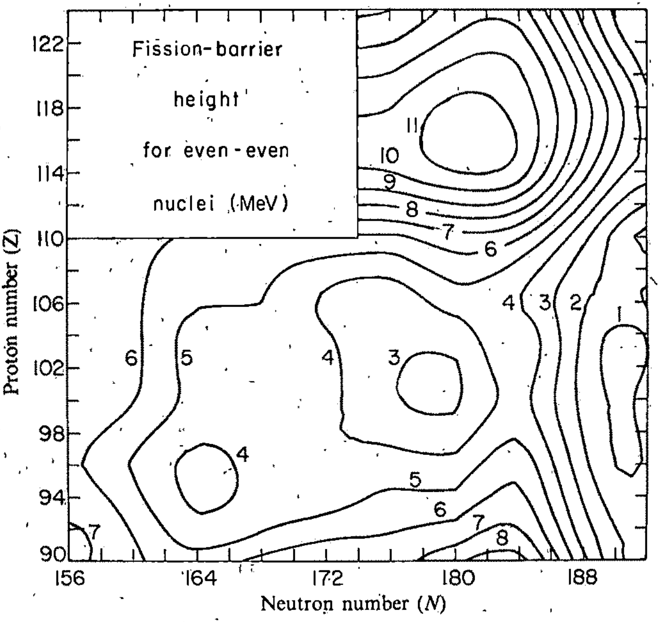

Fig. 2 Contour plot of the calculated fission-barrier height as a function of neutron number N and proton number Z for even nuclei. These fission barriers are calculated with droplet model constants that optimally reproduce nuclear ground state masses and fission barrier heights.

In order to study the sensitivity of the calculated barrier heights to the parameters of the droplet model, we have repeated the fission barrier calculations with two different sets of droplet model constants (W. D. Myers, private communication). These two sets of constants both give a standard deviation between the experimental and calculated ground state masses and fission barrier heights that is 10% larger than the optimum value. The results for these two different sets of droplet model constants are presented in contour plots in Figs 3 and 4. The constants used in the calculation in Fig. 3 yield an effective surface-asymmetry constant for ^{280}Pu of

$\kappa_{eff}=2.6$, while the constants used in the calculation in Fig. 4 yield $\kappa_{eff}=3.3$. As expected, the fission-barrier heights near the neutron closed shell at $N=184$ are almost independent of the droplet model parameters. The same microscopic corrections have been used for all of the calculations. In our consideration of the sequence of shapes for fission, we have not taken into account mass-asymmetric or axially asymmetric distortions. For some nuclei these effects could lower the calculated barriers by a few MeV.

Neutron Separation Energies

Figure 5 is a contour plot of the calculated neutron separation energy for even nuclei. The neutron separation energies decrease monotonically for increasing neutron excess. For a given determination of the neutron separation energy for neutron-rich nuclei, the r-process path can be determined approximately without carrying out a full r process calculation. Because it is a process in nuclear statistical equilibrium, it follows approximately a line of constant neutron separation energy, whose value we determine to be $B_n=3\pm1$ MeV for even nuclei.

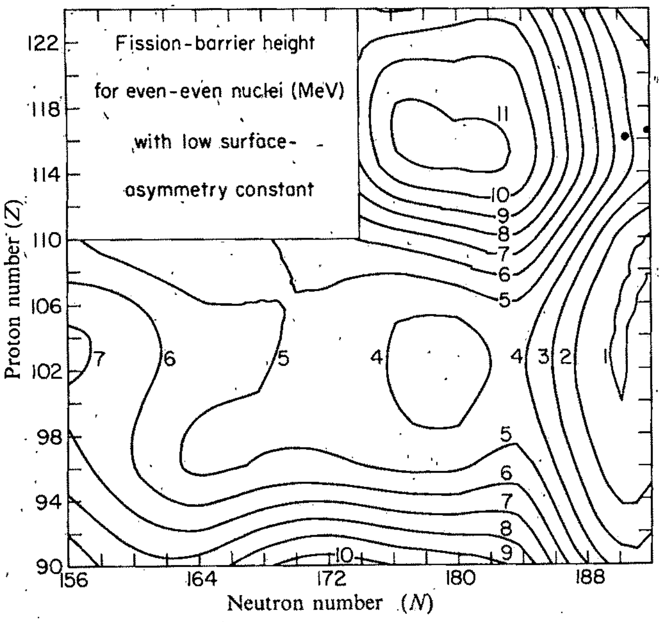

Fig. 3 Analogous to Fig. 2, except calculated with droplet model constants for which the effective surface asymmetry constant is lower. The error in the calculated ground state masses and fission barrier heights is 10% larger with these constants than with those used in Fig. 2.

Termination of Multiple Neutron-capture Processes

Figure 6 presents the essence of our results. When a nucleus captures a thermal neutron, it is excited to an energy equal to its neutron separation energy. When this energy is above the fission barrier height, then the nucleus will undergo neutron-induced fission at a much more rapid rate than the other available decay modes. Figure 6 is a contour plot of the difference between the fission barrier height and the neutron separation energy for even nuclei. From a study of Figs 5 and 6, we find the neutron-induced fission cutoff for the r process to be at $Z=96$ and $N=186$. Since the fission barrier heights in this region are almost independent of the macroscopic energy contributions, this cutoff is strongly dependent on the single-particle effects near the closed shell at $N=184$.

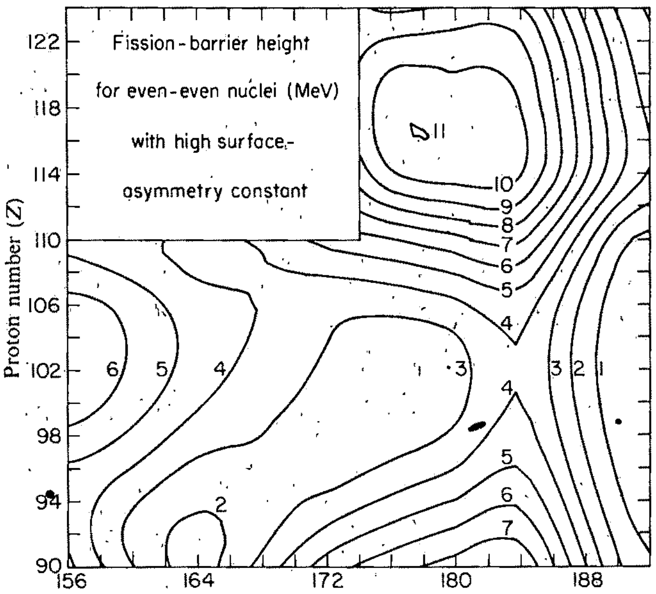

Fig. 4 Analogous to Fig. 2, except calculated with droplet model constants for which the effective surface asymmetry constant is higher. The error in the calculated ground state masses and fission barrier heights is 10% larger with these constants than with those used in Fig. 2.

Thus, we predict that nuclei with mass number $A=281$ will be the last to survive in the r process before neutron-induced fission terminates the path. According to the results of ref. 4, nuclei with mass number 281 would spontaneously fission with halflives of the order of 1 s after a few beta decays. In order for superheavy nuclei to be observed in cosmic rays, their halflives would need to be at least 10^6 yr. This would require the production of nuclei with mass number $A\gtrsim291$, which is 10 mass units higher than the heaviest yield that we predict.

Schramm and Fiset[13] suggest that a small fraction of the material at $N=184$ could climb along this closed shell during

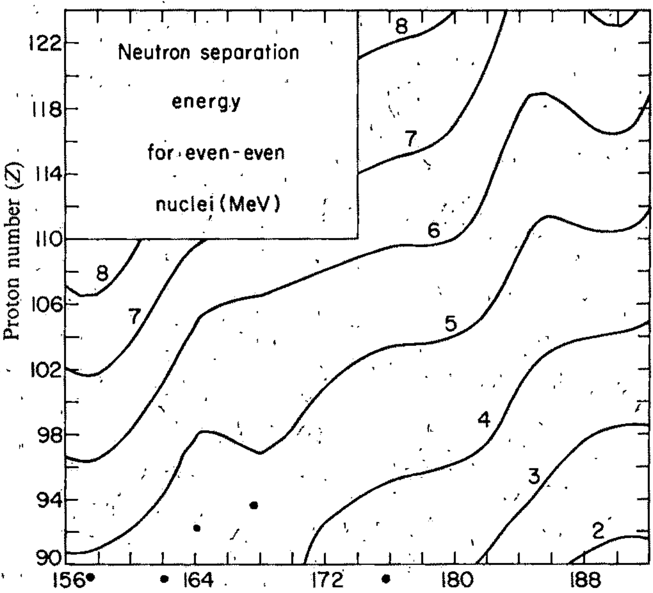

Fig. 5 Contour plot of the calculated neutron separation energy as a function of neutron number N and proton number Z for even nuclei.

a weak freeze out of the r process neutron flux and thereby reach the superheavy island. But as seen in Fig. 6, such nuclei would reach the 'cape' close to $Z=104$ and suffer neutron-induced fission before reaching the superheavy island.

Conventional nuclear explosions yield neutron exposures similar to those in the astrophysical r process; however, the time duration is so short ($\Delta t \lesssim 10^{-6}$ s) that there is no time for beta decays during the neutron-capture process. The thermal photon temperature is much less than in the r process. The neutron captures therefore proceed to a lower neutron separation energy, which extends into a more neutron-rich region. In nuclear explosions to date, targets of various actinide nuclei have been irradiated with intense neutron sources. Independent of the target used or the intensity of the neutron irradiation, the heaviest nucleus recovered from the debris[27] has been ^{257}Fm.

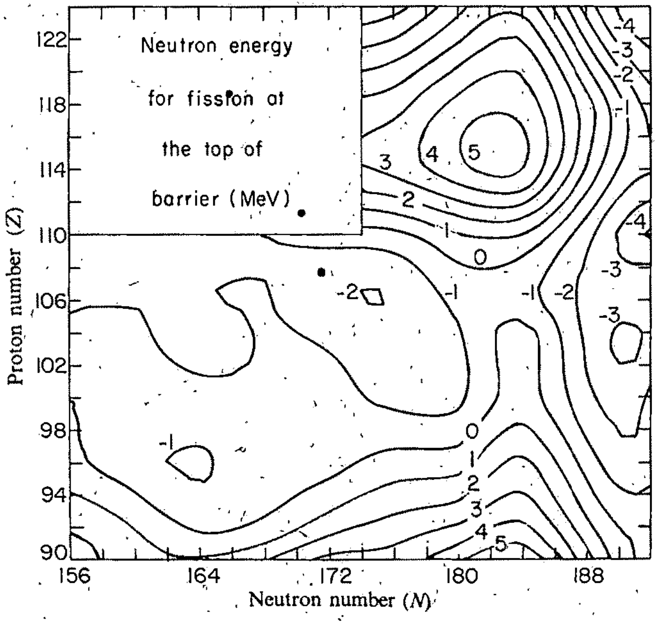

Fig. 6 Contour plot of the difference between the fission barrier height and the neutron separation energy for even nuclei.

We can understand this failure to produce heavier nuclei in terms of our results. From Fig. 6 we see that neutron-induced fission terminates the neutron capture chain on the U isotopes at mass number $A=256$. Actually, neutron-induced fission should occur somewhat before this because we overestimate the barrier for U isotopes in this region by 1 or 2 MeV by not including mass-asymmetric distortions. Thus, we conclude that conventional nuclear explosions have even less hope for producing superheavy nuclei than the r process.

The failure of conventional nuclear explosions to produce superheavy nuclei has led Meldner[16] to propose a multistep explosive process that would allow beta decays between subsequent explosions. But his process seems fraught with the same difficulties encountered in the r process and in conventional nuclear explosions. Certainly odd-mass capture chains would have to be utilised as well as a target lighter than Th to avoid initial termination of the process by neutron-induced fission. It would then be required that beta decay lead to a region of stability against neutron-induced fission before the next neutron burst.

Meldner would like to take advantage of neutron capture along odd-proton chains where the fission barriers could be enhanced relative to the barrier heights of even nuclei. In the actinide region the spontaneous-fission halflives of odd-particle nuclei are systematically about 10^3 times as long as those of neighbouring even nuclei[28]. These hindrances arise from an increase either in the height of the barrier or in the inertia (or in both). If this hindrance factor is assumed to arise only from an increase in the barrier height, then the barriers for odd-particle nuclei are raised by about 0.5 MeV relative to those for even nuclei[1].

An examination of Fig. 6 reveals that after an initial neutron burst and subsequent beta decay into $162 \lesssim N \lesssim 178, 92 \lesssim Z \lesssim 106$, the nuclei would be in a region where a second neutron burst would initiate neutron-induced fission. This conclusion also applies for odd-proton capture chains. But as we have seen, the relatively strong dependence of the fission-barrier heights in the region of $162 \lesssim N \lesssim 178$, $92 \lesssim Z \lesssim 106$ on the value of the effective surface-asymmetry constant makes this conclusion somewhat uncertain.

To summarise, we have used an improved version of the macroscopic–microscopic method to calculate fission barriers and neutron separation energies for a broad region of heavy neutron-rich nuclei. On the basis of these calculations we conclude that neutron-induced fission terminates the three possible multiple-neutron-capture processes well before the superheavy island is reached.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Model for Cygnus X-3

CYGNUS X-3 has been identified[1] with the infrared object found by Becklin *et al.*[2] to be within ±2″ of the radio position: the infrared object is found to vary in intensity with the same period as and in phase with the X-ray object. The infrared flux (2.2 μm) varies smoothly by about 15% whereas the X-ray intensity varies by a factor ~3. Cyg X-3 is unique among other regularly varying sources in that the X-ray light curve is smooth and roughly sinusoidal[3], displaying no sudden intensity variations indicative of an eclipse of a compact X-ray source by a larger object (for example, a companion star). We show that nevertheless Cyg X-3 can be interpreted by means of the 'standard model' for galactic X-ray sources in terms of mass loss from a large companion star and accretion onto a compact object.

We assume throughout that the 4.8 h period is due to orbital motion. Kepler's Law (assuming a circular orbit) yields

$$D \approx 10^{11}\ (M/M_\odot)^{\frac{1}{3}}\ \mathrm{cm} \qquad (1)$$

where D is the distance between the two stars and M is the total mass of the system. If this is also the size of the infrared object (or an upper limit to it) we can obtain a lower limit to the brightness temperature at 2.2 μm (see ref. 1)

$$T_B \gtrsim 3\times10^6\ (M/M_\odot)^{-\frac{2}{3}}(d/10\ \mathrm{kpc})^{\frac{1}{2}}\ \mathrm{K} \qquad (2)$$

where d is the distance to the source. From radio data[6] we know that $d \gtrsim 10$ kpc.

If the bulk of the infrared flux is due to radiation from the surface of an ordinary star we need[4] $M \gtrsim 70\ M_\odot$. In this case the 2.2 μm variation cannot be due to tidal effects—this would give two infrared minima per X-ray period—and the most likely explanation would be that it is a reflection effect. For a 15% variation in the infrared, the X-ray source must give a variation in the energy flux over the stellar surface of nearly a factor of two. This is possible, but, if so, the reflection effect could easily give rise to all the infrared emission, obviating the need for such a massive system. I shall therefore assume the standard model: X rays originate from a compact object (I show below how the light curve may be produced) and the infrared flux is due to heating of the companion star (or its extensive atmosphere or wind) by the X-ray star.

The observed mean X-ray luminosity in the 2 to 6 keV range is[5]

$$L_X \approx 3\times10^{37}\ (d/10\ \mathrm{kpc})^2\ \mathrm{erg\ s^{-1}} \qquad (3)$$

If the infrared emission comes from the Rayleigh-Jeans tail of radiation from an optically thin source emitted by a gas at temperature T_B, the total luminosity of the gas is given by

$$L_g \gtrsim 4\times10^2\ L_{IR}(M/M_\odot)^{-\frac{2}{3}} \qquad (4)$$

where L_{IR} is the luminosity at 2.2 μm. Using the dereddened flux at 2.2 μm (ref. 1)

$$L_X \approx 4\times10\ L_{IR} \qquad (5)$$

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Thus the total X-ray luminosity must be given by $L_{XT} = f\ L_X$ where

$$f \gtrsim 10(4\pi/\Omega)(1-\alpha)^{-1}(M/M_\odot)^{-\frac{2}{3}} \qquad (6)$$

Ω is the solid angle subtended by the gas from the X-ray source and α is the albedo. Thus to power the infrared source we require a total X-ray luminosity in excess of that observed in the 2 to 6 keV range. That is, the bulk of the X-ray luminosity is emitted either above 6 keV or below the low energy cut-off at about 3 keV. This is similar to what is required in the system Her X-1/HZ Her (ref. 7 and Y. Avni *et al.*, to be published). If the inclination of the orbit i is moderate (say $30° \lesssim i \lesssim 60°$), variation in aspect of the gas and of the amount of gas visible can easily produce the required smooth 15% variation.

I now outline a possible model for producing the X-ray light curve. I suggest that the smooth variation of the X-ray luminosity is produced by a gradual and partial eclipse of the (compact) X-ray object by a stellar wind emanating from its larger companion. This same wind can also give rise to the mass transfer required to power the X-ray source. The 2 to 6 keV spectrum has a variable low energy cut-off, but this variation does not appear to be correlated with the 4.8 h period (P. Sanford, private communication). Thus, and also for simplicity, I assume that the main opacity in the wind is electron scattering. For moderate inclination i we shall see that the electron scattering optical depth τ_{es} can vary by order unity over the orbital period. Thus if $\tau_{es} \sim 1$, the luminosity of the compact source will seem to vary by ~3. If, however, absorption is unimportant everywhere in the wind, backscattering of X rays would provide a diffuse background X-ray luminosity comparable to that of the compact object. So to obtain the required X-ray variation, we need absorption to be important but not dominant.

For the sake of argument I limit discussion to a specific model of the wind. I assume (see ref. 7) that the wind is emitted by the star in a spherically symmetric fashion at about the escape velocity, and that the parts of the wind where absorption and scattering take place are dynamically unaffected by the presence of the X-ray source. Conservation of mass implies that the wind density falls off as the inverse square of the distance from the star. The electron scattering optical depth is given as a function of θ, the angle between the line of sight and the axis of symmetry of the system by

$$\tau_{es} = 0.2\times10^{-24}\ NDg(\theta) \qquad (7)$$

where

$$g(\theta) = (\pi-\theta)/\sin\theta \qquad (8)$$

and N is the number density at distance D from the large star. The unscattered X-ray flux seen at infinity, L_U, is given in terms of the source luminosity L_S by

$$L_U = L_S \exp\{-\tau_{es}(\theta)\} \qquad (9)$$

and the X-ray light curves obtained using this are shown in Fig. 1 for various values of the inclination. For each value of i, $\tau_{es}(\pi/2)$ has been chosen such that the overall luminosity variation is by about a factor of three. Note the similarity between these curves and the actual light curve of Cyg X-3.

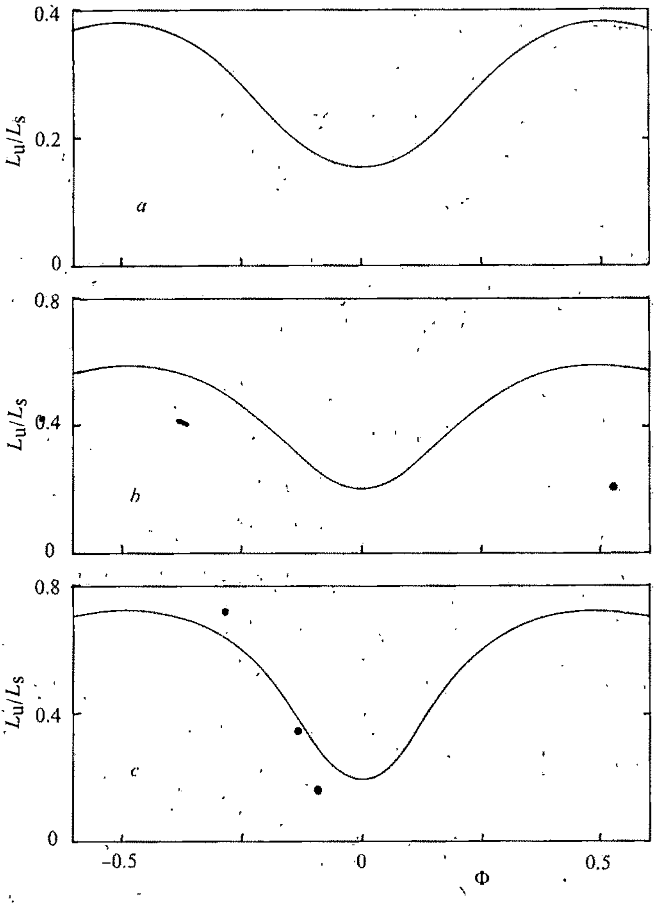

Fig. 1 The fraction of X-ray flux that reaches the observer unscattered, L_U/L_S, is plotted against binary phase Φ for various values of the inclination i and the optical depth to electron scattering $\tau_{es}(\pi/2)$. *a*, $i=30°$, $\tau_{es}(\pi/2)=1.25$; *b*, $i=45°$, $\tau_{es}(\pi/2)=0.75$; *c*, $i=60°$, $\tau_{es}(\pi/2)=0.5$. For each i, the value of $\tau_{es}(\pi/2)$ has been chosen to give an overall variation of about a factor of three.

I argued earlier that absorption in the wind must be important but not dominant. That is, we must have

$$\lambda = F/(4\pi D^3 N^2 a) \simeq 1 \qquad (10)$$

where F is the ionising photon flux and a the effective recombination coefficient (see ref. 7 for a fuller discussion). Using $a \sim 3\times10^{-10}\,.\,T^{-\frac{3}{4}}$, we obtain

$$N \approx 10^{13}(M/M_{\odot})^{-\frac{1}{2}} L_{38}^{\frac{1}{2}} T_6^{\frac{3}{8}}\ \mathrm{cm}^{-3} \qquad (11)$$

where L_{38} is the X-ray luminosity in units of 10^{38} erg s^{-1}, T_6 is the temperature of the wind in units of 10^6 K and I have assumed the typical ionising photon to have an energy of 5 keV. This density implies

$$\tau_{es}(\pi/2) \approx 0.5(M/M_{\odot})^{-\frac{1}{6}} L_{38}^{\frac{1}{2}} T_6^{\frac{3}{8}} \qquad (12)$$

in rough agreement with previous estimates. This density also indicates that the overall mass loss rate from the large star must be

$$\dot{M}_1 \approx 10^{-6}(M/M_{\odot})^{\frac{1}{2}}(M_1/M) L_{38}^{\frac{1}{2}} T_6^{\frac{3}{8}}\ M_{\odot}\ \mathrm{yr}^{-1} \qquad (13)$$

where M_1 is the mass of the large star. So for this model we expect the 4.8 h period to change on a time scale of ~10^6 yr.

I note also that inhomogeneities in the wind and variations of the wind strength can lead to irregular variations in the low energy cut-off. Absorption is more important near X-ray minimum, and so the X-ray curves calculated on the basis of electron scattering alone will be distorted downwards near minimum. Thus the variation will be less pronounced at high energies than at low energies where absorption is more important. I note in passing that a mass loss rate of ~$10^{-3}\ M_{\odot}$ yr^{-1} of the kind suggested by van den Heuvel and De Loore for the source Cen X-3 (ref. 8) would entirely extinguish any X-ray flux from the compact object. Unless the velocity of the wind exceeds the escape velocity from the system by several orders of magnitude, the wind density required would imply $\tau_{es}(\pi/2) \gg 1$ and $\lambda \ll 1$.

This model predicts that a large fraction of the total X-ray luminosity is absorbed in the stellar wind. Basko and Sunyaev[9] show that for stars with a high mass loss rate most of the energy is absorbed by gas with $T \sim 10^6$ K (comparable to T_B). Most of this energy is re-radiated as ultraviolet and as soft X rays from a region whose size is comparable to that of the system. This confirms that the spectrum from the gas will be flat out to wavelengths as long as 2.2 µm, before turning over to Rayleigh-Jeans at longer wavelengths. If this soft X-ray flux were visible we expect it to vary in a similar way to the infrared flux. The hard X-ray flux varies by a much greater amount and so cannot come from the same region. Also, the optical flux predicted here is much less than that expected from a straightforward extrapolation of the infrared flux along the Rayleigh-Jeans curve, as would be the case if the bulk of the infrared flux came from the stellar surface. Finally, I stress again the uniqueness of the system Cyg X-3 and, in a sense, the uniqueness of this model. For the parameters of Cyg X-3 we can have $\tau_{es} \sim 1$ and $\lambda \sim 1$. If the system were larger or smaller we could not satisfy both of these for any wind density, since $\lambda\tau_{es}^2 \propto D^{-1}$, independent of N.

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Close Pairs of QSOs

IT has recently been argued[1,2] that the observation of close pairs of QSOs with very different redshifts provides support for non-cosmological explanations of the redshifts. We show in this note that the pairs observed so far may be comfortably explained as random coincidences and that the small probabilities that are often quoted depend on the use of *a posteriori* statistics.

The expected number of close pairs of QSOs is given by

$$\mathscr{N} = N_Q\ (\pi R^2)\ \rho(\leq m) \qquad (1)$$

where N_Q is the total number of QSOs whose redshifts have been determined, R (in arc s) is the 'region of interest' around any given QSO (or the maximum allowable separation for 'close' pairs), and $\rho(\leq m)$ is the total number of QSOs per square arc s down to a limiting magnitude m. Equation (1) may be rewritten inserting typical numbers[3], as

$$\mathcal{N}(R,m) \approx 7 \times 10^{-2}\ (N_Q/1{,}000)\ (R/10'')^2\ 10^{0.6(m_v-19)} \qquad (1')$$

It should be noted that the typical *a posteriori* probabilities, calculated with the aid of equation (1), for the chance occurrence of a pair of close QSOs are not terribly small; they roughly correspond to 3 σ events.

We recall the basic observational facts. There are now two known 'close' pairs of QSOs: Ton155, 156 (ref. 1) with a separation of $35''$, redshifts $z = 1.7$ and 0.55, and $m_v \approx 16.0$ and 16.6 mag; and 1548+115 a, b (ref. 2) with a separation of $5''$, redshifts of $z = 0.44$ and 1.9, and $m_v \simeq 17$ and 19 mag.

We note that the initial observation[1] of a close pair of QSOs, Ton155 and Ton156, could not be regarded by itself as statistically significant because the typical correlation-separation previously discussed[4] was several degrees (yielding for typical values of R and m in equation (1) values of $\mathcal{N} \gg 1$). But we might legitimately have used the initial observation to define an hypothesis to be tested, for example: QSOs brighter than $m_v = 17$ mag have a much higher chance of appearing close together, $R \leq 35''$, than would be expected from a random distribution. On the basis of this hypothesis, the subsequent observation of the close pair 4C11.50 a, b (= 1548 and 115 a, b) is not remarkable. First, m_v (1548 and 115 b) is ~ 19 mag, much fainter than either of the Ton objects, so that the above defined hypothesis does not even cover this case. If we extend the limiting magnitude down to 19.4 mag (so it comfortably covers the observed pair 4C11.50 a, b), then $P(35'', 19.4) \sim 0.5$ compared with the observed value of 2. Actually even this calculation underestimates the expected number of pairs on the cosmological hypothesis. There is no *a priori* reason to suppose that $m = 19$ mag or even $m = 19.4$ mag is especially significant and one will always get unrealistically small probabilities for events if one calculates after the fact the chance expectation using the already observed parameters to define the experiment. In fact, experience shows that *a posteriori* probabilities at the 1% level are not particularly uncommon for random events; but of course *a posteriori* probabilities at the 10^{-6} or 10^{-10} level still are.

It is interesting to estimate the number of close QSO pairs that one would expect *a priori* on the cosmological hypothesis down to the limiting magnitude (~ 21 mag) of the blue Sky Survey (on which most of the identifications are made). For a total of 10^{-3} QSOs with known redshifts (perhaps a reasonable expectation for the next several years) and a maximum separation of $10''$, we expect one or possibly a few pairs. If we enlarge the maximum separation to $30''$ we expect about ten close pairs. These estimates are of course uncertain by at least a factor of three because we do not yet have an accurate determination of the QSO spatial density at faint magnitudes. Nevertheless, close pairs of QSOs should be rather common when it becomes standard practice to measure redshifts for all faint stars near accurate radio source positions.

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Response of Dayside Thermosphere to an Intense Geomagnetic Storm

INCREASES in the density of the neutral thermosphere associated with geomagnetic activity were first noted by Jacchia[1]. Although the thermosphere will not be in equilibrium, and acceleration by electrodynamic forces may be involved, on balance the increased density must reflect additional heating. Further studies[2-4] by orbital drag analysis from observations of artificial Earth satellites have led to a complex and somewhat confusing picture. The heating seems to be slightly greater[3,4] at high latitudes and, for larger disturbances, sometimes much greater[2,3]. Also, the heating seems to be slightly greater[3,4] on the nightside, but possibly with the opposite trend[3] for larger disturbances ($K_p > 5$). Jacchia *et al.*[3] also found that the time delay between the peak of the storm, as measured by the planetary 3-h indices K_p or a_p, and the maximum response was a little shorter at high latitudes; on the other hand Roemer[4] concluded that the time delay was independent of latitude, height or local time.

The low g accelerometer calibration system (LOGACS) experiment[5,6] carried on an attitude-controlled Agena vehicle launched by the United States Air Force on May 22, 1967, provided a unique opportunity to determine the response to an intense storm. As the histogram in Fig. 1 shows, the planetary 3-h geomagnetic index, a_p, started to increase on May 25, reached a peak of 236, then fell slightly before increasing to the conventional maximum of 400 for two successive 3-h periods. Figure 1 also gives the hourly equatorial Dst index[7] which is based only on the horizontal H components at low latitude observatories and effectively measures the strength of the ring current during the storm.

The LOGACS accelerometer provided values of atmospheric density with an estimated accuracy better than 10% and a time resolution of 1 s, although at much longer intervals. Using these values, DeVries[5,6] found that the density in the (northern) nightside auroral regions increased with the onset of geomagnetic activity whereas the density in the (northern) dayside auroral regions initially decreased. The density over the rest of the Earth apparently reached a maximum with a time delay ranging from near zero in the nightside auroral regions to about 6 h at the dayside equator. DeVries concluded that most of the energy was injected in the nightside auroral regions probably, he suggested, by Joule heating[8] at or below 150 km, and transported over the rest of the Earth by convective circulation and atmospheric gravity waves.

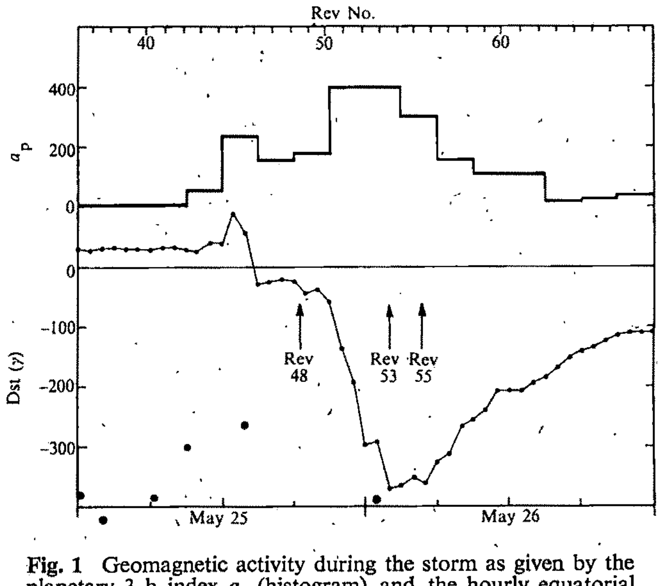

Fig. 1 Geomagnetic activity during the storm as given by the planetary 3 h index a_p (histogram) and the hourly equatorial Dst index. Rev, revolution.

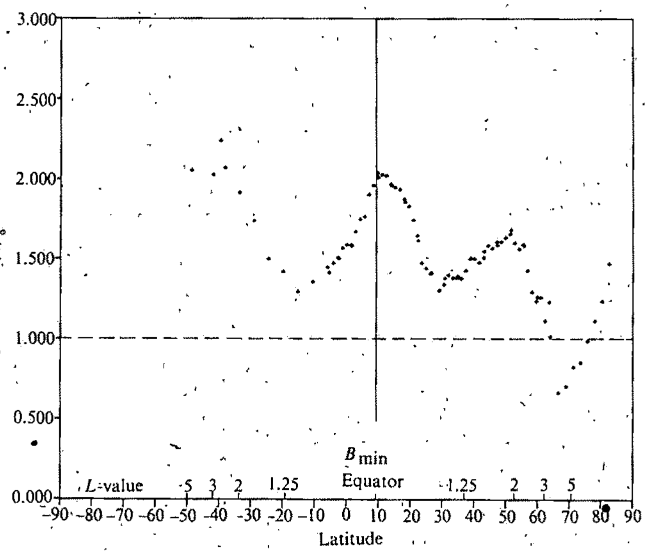

Fig. 2 The density ratio ρ/ρ_0, where ρ_0 is a standard quiet-time density, for the dayside pass on revolution 53 in the LOGACS experiment. The height of the satellite, which is near polar and passes southwards down the dayside at 1030 LT, is 165 km at 80° N falling to a minimum of 140 km at 40° N; then rising to 165 km at the equator and 305 km at 80° S. The magnetic equator and the feet of various L shells are also shown.

At the invitation of DeVries, we have made an independent analysis (R. R. A. and G. E. Cooke, unpublished) of the LOGACS data in which we concentrated on the dayside passes as these are more complete and contain the most clearly defined structures. The orbit is near-polar with the north-going passes on the nightside at about 2230 LT, and the south-going passes on the dayside at about 1030 LT. Perigee, at a height of 140 km, occurred on the dayside near 45° N, and the apogee height was between 300 and 400 km. For the dayside passes the response in both hemispheres can be compared, whereas on the nightside the vehicle is above 300 km, where the density values are much sparser and less reliable, south of the equator. The most clearly developed dayside response appears on revolution 53 just as the Dst index reaches its negative peak. The response is shown in Fig. 2 in the form of the ratio of the density on revolution 53 to a standard quiet-time density ρ_0, which has been derived (R. R. A. and G. E. Cooke, unpublished) from all the quiet-time passes up to revolution 41. Unfortunately, there is a gap in the latitude coverage from 50° S to 80° S in Fig. 2, but revolution 55 is essentially complete and is shown in Fig. 3.

The two figures are very similar; excepting the gap in Fig. 2, they show a wave-like pattern with a central maximum at 10° N, further maxima at 50° N and 40° S, and yet further maxima approaching the polar caps. The pattern is more nearly symmetrical (in form if not in magnitude) in magnetic coordinates, with the central peak on the magnetic equator and the mid-latitude peaks near $L=2$ in the Northern Hemisphere but perhaps nearer $L=3$ in the Southern Hemisphere. This slight asymmetry could result from the greater height in the Southern Hemisphere; alternatively the geometry could be imposing a compromise between geographic and geomagnetic latitudes. On the other hand, the greater magnitude of ρ/ρ_0 in the Southern Hemisphere probably arises simply from the greater height.

Although the gross structure of the response has a wave-like pattern, it is not a propagating wave. The dayside pass on revolution 54 is incomplete, but what there is shows the same latitude dependence and densities intermediate between revolution 53 and revolution 55. There are certainly small-scale wave-like features, such as the double peak at 50° N in Fig. 3, but I hesitate to interpret them as evidence for atmospheric waves.

The appearance of pronounced troughs near ±60° magnetic latitude is in complete agreement with DeVries[5,6]. The northern trough appears in both figures and reveals a minimum density of only 70% of the quiet-time value (at a height of 150 km) on revolution 53, rising to 85% by revolution 55. Two possible reasons might be that the air has been accelerated and displaced by electrodynamic forces or that the air is colder, due to the displacement of part of its normal heat source to lower latitudes.

The response time of the dayside thermosphere must be fairly short because the density at the peaks is at, or nearly at, its maximum on revolution 53. At the mid and high-latitude peaks the density perhaps increases marginally by revolution 55. The equatorial peak, however, has nearly disappeared by revolution 55, possibly because ion drag can offer negligible resistance to an expansion in latitude. On the other hand, the density between the peaks continues to increase for some hours. Eventually the structure collapses in irregular fashion —so irregularly that it might be said to disintegrate. The rapidity of the response and its approximate symmetry in magnetic coördinates indicate that the structure shows simply where the energy is injected into the atmosphere, at least in the morning sector, in an intense storm, namely, near the magnetic equator, near the particle precipitation zones which are displaced equatorwards in the storm and over the polar caps.

Moreover, the peak values of ρ/ρ_0 at the greater heights in the Southern Hemisphere exceed the corresponding peaks in the Northern Hemisphere and seem to be attained just as rapidly. The implication seems to be that a significant part of the energy input must raise the temperature fairly uniformly throughout the height range and is therefore possibly due to particle precipitation rather than Joule heating.

The most interesting feature of the response is the pronounced peak on the magnetic equator on revolution 53. As this pass coincides with the maximum negative excursion (−370 γ) of the Dst index, the obvious conclusion is that the equatorial peak is related to the growth and decay of the storm-time ring current. This conclusion is reinforced (R. R. A., unpublished) by the appearance of a similar, if less marked, peak on revolution 48 soon after the first negative excursion of Dst (Fig. 1). As has been pointed out[9–11], an important loss process for ring current protons will be the charge-exchange collision with neutral hydrogen atoms. The collision results in a fast neutral hydrogen atom which is later stripped at low altitudes (if it goes downwards) to recover a proton, which is then trapped until a third atmospheric interaction removes it, and so on. Subsequently other loss mechanisms, pitch-angle

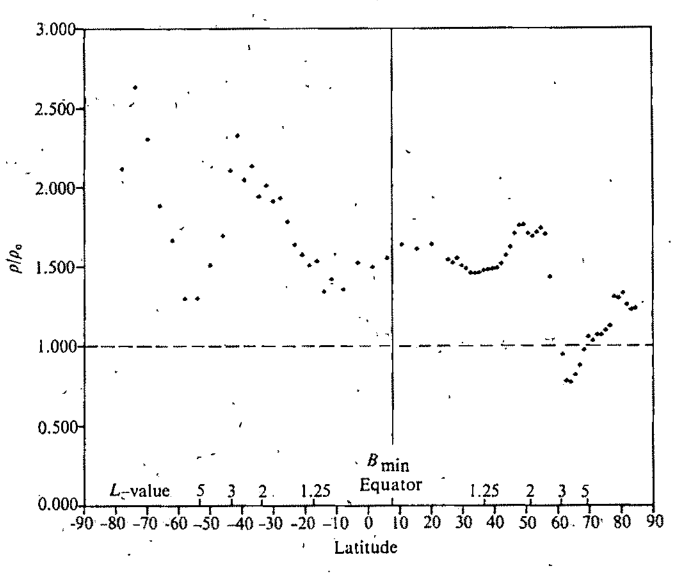

Fig. 3 The corresponding density ratio for the dayside pass on revolution 55, with the details as for Fig. 2.

diffusion due to Coulomb scattering or plasma instabilities, have been extensively discussed and the charge-exchange collision has been somewhat overlooked. Recently, however, new calculations[12] using data on hydrogen densities in the geocorona show that the charge-exchange collision ought to be the dominant loss mechanism below $L=4$. For a large storm, the ring current energy will typically[13-15] peak somewhat above $L=3$ with a sharp cutoff below $L=3$.

This interpretation of the equatorial peak is confirmed by recent particle measurements below $L=1.15$, which is the lower limit for orbits which can drift completely around the Earth. Moritz[16] finds an ever-present flux of protons near the magnetic equator which he interprets as resulting from charge-exchange losses from the quiet-time ring current. The flux is constant at quiet times (which is consistent with both production and loss processes being proportional to atmospheric density) but increases by up to a factor of three during geomagnetic activity; in fact the energy range (250 keV to 1.65 MeV) of the detector is well above the energy (5 to 50 keV) typical[13,14] of storm-time ring current protons. More extensive results[17] are now available from OV1–17 for the energy range 12.4 to 500 keV at about 400 km near the magnetic equator at local midnight. The flux of protons below 100 keV increases sharply at disturbed times, but above 200 keV the flux is relatively stable and in agreement with the AZUR results[16]. Mizera and Blake[17] find that the flux near 40 keV is particularly well correlated with the growth of the ring current.

If the interpretation is correct, the existence of a response sharply peaked about the equator allows one interesting deduction. At this height (~ 160 km) the lifetimes of any charged particles produced by the precipitating neutral hydrogen atoms must be much shorter than their bounce periods so that the response must reflect the energy input. For a dipole field line $r=L\cos^2\Lambda$, the centre of curvature at the equator lies at $2L/3$. Thus, the narrowness of the response cannot result from a geometrical focusing effect unless the source lies below about $L=2$, which seems unlikely, or unless the field is grossly distorted by the currents flowing. It therefore seems more probable that the part of the storm-time ring current responsible for the equatorial peak must be confined fairly closely to the equatorial plane. In support of this view, Frank[14] has noted that his observations "strongly suggest that these protons are injected into the outer radiation zone as an 'equatorial plasma jet' during the initial phase of a magnetic storm". Similarly, Sugiura[18] finds that the quiet-time ring current is closely confined to the equatorial plane.

The near disappearance of the equatorial peak by revolution 55 also calls for further comment. One possible reason would be that the storm-time ring current particles have acquired significant bounce amplitudes, so that the energy is being deposited over a wider latitude band. It seems more likely, however, that the closest part of the ring current has been removed by revolution 55, in line with the estimated[12] charge-exchange lifetime of 3 to 10 h at $L=3$. For a storm of magnitude $-150\ \gamma$ on April 17, 1965, Cahill[13] finds that a large part of the energy between $L=2.5$ and $L=3.5$ (estimated to be $\sim 10^{21}$ erg) is lost in less than 2 h.

The interpretation of the equatorial response as resulting from charge-exchange losses from the ring current is also in agreement with the results of May and Miller[19] on the response of the equatorial atmosphere (at about 310 km near noon local time) from the decay of the spin rate of Ariel 2 in March and April 1964. They noted that the density variations were more closely correlated with Dst than with a_p.

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African Swells, Magmatism and Plate Tectonics

WRIGHT[1,2] has revived Bailey's lateral compression model[3] for the generation of Africa's topographic swells[4-6] and the related igneous activity. Here I discuss some flaws in this approach, many of which persist in spite of its translation into plate-tectonic language.

Bailey regarded the gross "basin and swell" topography of Africa as the result of lateral compression of the continent, and he suggested that such regional updoming of the crust can bring about sufficient relief of lithostatic loading to cause melting in the deep crust (or below), to produce the characteristic East African igneous activity. Wright's variant[1] attempts a marriage between this scheme and modern global tectonics: here the entire lithosphere plate undergoes buckling, which is attributed to lateral stress attending opposed spreading at the mid-Atlantic and mid-Indian Ocean ridges[2].

To propose that pressure relief in the deepest parts of the crust is responsible for melting[3] clearly requires that the crust alone should suffer warping, the layer beneath being relatively free of lateral stress and deformation: pressure relief can only occur at the junction between deformed and undeformed layers. Such a suggestion implies a degree of decoupling between the crust and underlying lithosphere at odds with modern global tectonics, which emphasises the integrity of the entire lithosphere layer, and it is hard to think of a mechanism by which the crust alone could be subject to compression.

If, on the other hand, one considers that the lithosphere layer as a whole undergoes buckling[1], similar arguments restrict the possibility of load relief and melting to the base of the plate, at the lithosphere-asthenosphere interface. To suggest that significant pressure relief can be induced here by plate warping assumes an order of rigidity in the asthenosphere which is questionable. Fennoscandian and Hudsonian postglacial uplift rates are known to be of the order of 1 cm yr^{-1} (ref. 8). It seems, therefore, that asthenosphere flow can be rapid enough to compensate a lithosphere uplift taking place at this rate, which is nearly two orders of magnitude greater than the mean rate of doming uplift in East Africa (5,000 foot in $\sim 1.5\times 10^7$ yr according to ref. 4, page 1056). Since there is no evidence of deep partial melting associated with the postglacial relief of shield areas, one may argue that the probability of its occurring through compressional warping of similar terrains in Africa is insignificant. There is evidence that uplift in East Africa has been a discontinuous process, and it is arguable that intermittent spells of rapid uplift may have sustained melting, but the data above suggest that uplift rates 100 times greater than the observed average are required before melting is remotely possible. Such rates seem unlikely.

There is evidence too that lateral compression is not capable of producing Africa's swells. Ramberg and Stephansson[9] have analysed the effect of gravity on the maximum width of compressional warp structures, and have calculated an upper 'wavelength' limit of the order of 10 km. Comparison with the scale of the African features (200 to 500 km or more) points to the operation of some other mechanism. Moreover, the presence of tensional rift structures astride the swells belies the involvement of compression[10].

There are several petrological observations bearing on partial melting models. Bailey proposed that East Africa's abundant salic lavas derive from the partial fusion of deep-crustal rocks of alkali basalt affinity. Leaving aside the efficiency of pressure relief in producing melting[7], this idea fails to explain the coexistence at the surface of salic and basaltic volcanism, unless locally complete fusion is advocated. The origin of the basaltic reservoir and the reasons for its confinement to deep levels are unexplained.

Wright emphasises the mantle as the source of volcanism. There is, however, little petrological evidence that liquids as fractionated as trachyte or phonolite can form by fusion of ultramafic compositions. (If that were possible, why should the proportion of salic lavas be so much lower in oceanic islands than in the continents?) Rare high pressure xenoliths in trachytic rocks indicate high pressure fractionation[2] but not necessarily an origin by direct partial fusion of mantle rocks[11]. They are equally consistent with fractional crystallisation of bodies of mafic liquid confined near the base of the crust, a model which explains a variety of phenomena[12]. Moreover, any partial fusion scheme predicts that low-temperature melts such as trachyte should be sweated off from the mantle substantially earlier than basaltic liquids, a generalisation which is hard to reconcile with the simultaneous or alternating availability of both end members in many alkaline provinces.

I have assembled arguments showing that warping by lateral compression is at best an improbable cause of continental alkaline magmatism. The topography of Africa is now widely regarded as the product of heat flow anomalies in the upper mantle[6,7], from which the igneous activity where present may also be derived. I have argued recently[12] that the overabundance of salic magmas in the alkaline provinces of Africa and elsewhere, far from indicating highly specialised mechanisms for magma generation beneath the continents, merely reflects the influence of low-density continental crust on the transport of magmas of different types to the surface or present erosional levels. Thus the abundance anomaly which Bailey[3] and Wright[1] seek to explain may only be superficial, and there is little reason to adhere to a model of magma genesis which fits so few of the data now available.

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Behaviour of the Earth's Palaeomagnetic Field from Small Scale Marine Magnetic Anomalies

CERTAIN areas of the ocean crust exhibit a high resolution recording of the magnetic field history. A recent survey[1] of the Gorda-Juan de Fuca Rise area in the North Pacific displays short wavelength (10 to 20 km), low amplitude (40 to 80 gamma) features superimposed upon the larger scale (30 to 200 km, 200 to 800 gamma) magnetic anomaly pattern of Heirtzler *et al.*[2]. Many of these small scale anomalies form lineations which are parallel to the major magnetic lineations of Heirtzler *et al.* Sequences of small scale anomalies form characteristic patterns within intervals previously thought to be of constant polarity. We have identified two of these patterns that we observed in the North Pacific on profiles from the South Pacific and South-east Indian Oceans. Because of their global distribution, we conclude that the small scale anomalies are due to time variations of the Earth's magnetic field. That is, these features record either short (less than 3×10^4 yr) polarity reversals or fluctuations in the intensity of the dipole moment, perhaps with periods greater than 3×10^4 yr.

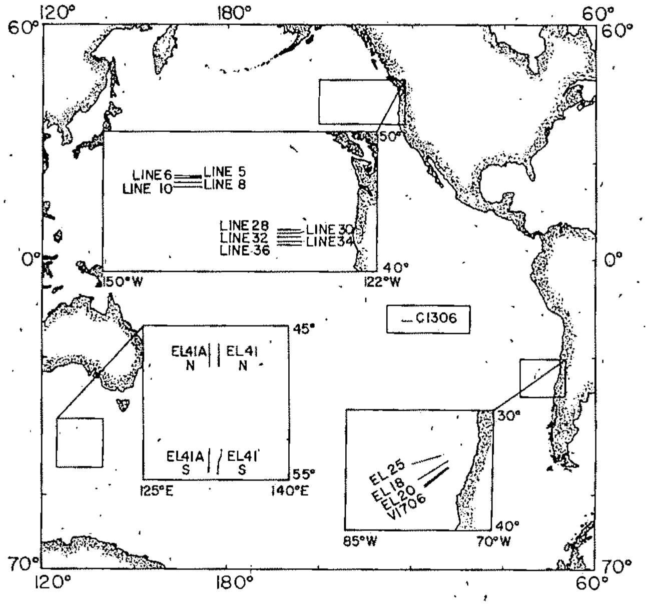

Fig. 1 Locations of profiles in Figs 2 and 3. Track prefixes: C, RV Conrad; V, RV Vema; EL, USNS Eltanin; LINE refers to Surveyor Seamap profiles.

The two sequences of small scale anomalies we studied in the North Pacific survey were between anomalies 12 and 13 (half-spreading rate 4.5 cm yr^{-1}) and between anomalies 5 and 5A (half-spreading rate 3.0 cm yr^{-1}): see Figs 2 and 3. On the east flank of the Chile Rise (half-spreading rate 4.5 cm yr^{-1}) the lineated small scale features between anomalies 12 and 13 show the same character as in the North Pacific survey area (Fig. 2). Similarly, the small scale features between anomalies 5 and 5A in the North Pacific can be recognised on magnetic profiles of both flanks of the South-east Indian Ridge (half-spreading rate 2.8 cm yr^{-1} on north flank; 2.3 cm yr^{-1} on south flank). These features can also be identified on the Conrad 1306 track (Fig. 3) which crosses anomaly 5 and 5A in the Central Pacific in an area of relatively fast spreading (half-spreading rate 6.0 cm yr^{-1}). This profile contains additional detail which may represent actual field behaviour recorded with higher resolution because of the fast spreading rate. It would be beneficial to conduct a survey of this area to determine whether this detail is repeated in other profiles.

To facilitate the correlations, we have phase shifted the various profiles according to the method of Schouten and McCamy[3]. The amount of phase shift is noted by the parameter Δθ in Figs 2 and 3.

The detailed behaviour of the Earth's magnetic field is not well known. The original reversal time scale of Heirtzler *et al.* identified no polarity events of duration less than 3×10^4 yr. Research on the magnetisation of rocks[4,5] and high sedimentation rate cores[6,7] however, suggests the existence of at least six short (less than 3×10^4 yr) polarity events during the past 2.4 m.y. Others[2,8,9] have correlated some of these events with small scale anomalies on marine magnetic profiles. Blakely and Cox[10] identified six additional short (less than 6×10^4 yr) polarity events in the early Cainozoic from magnetic profiles. Blakely[11] is revising the Heirtzler time scale between anomalies 5 and 6. He has identified many of the same small scale features that we have between anomalies 5 and 5A.

McElhinny[12] points out the possibility that some of the events identified from the magnetisation of rocks and high sedimentation rate cores may be due to polar excursions of the dipole field rather than complete polarity reversals. In addition, near-bottom magnetic surveys suggest that there are variations in the magnetisation intensity of the ocean crust due either to fluctuations in palaeomagnetic field intensity or variations in petrology[13,14]. Others[15,16] have noted the existence of dipole field intensity fluctuations with a periodicity of 10^4 yr.

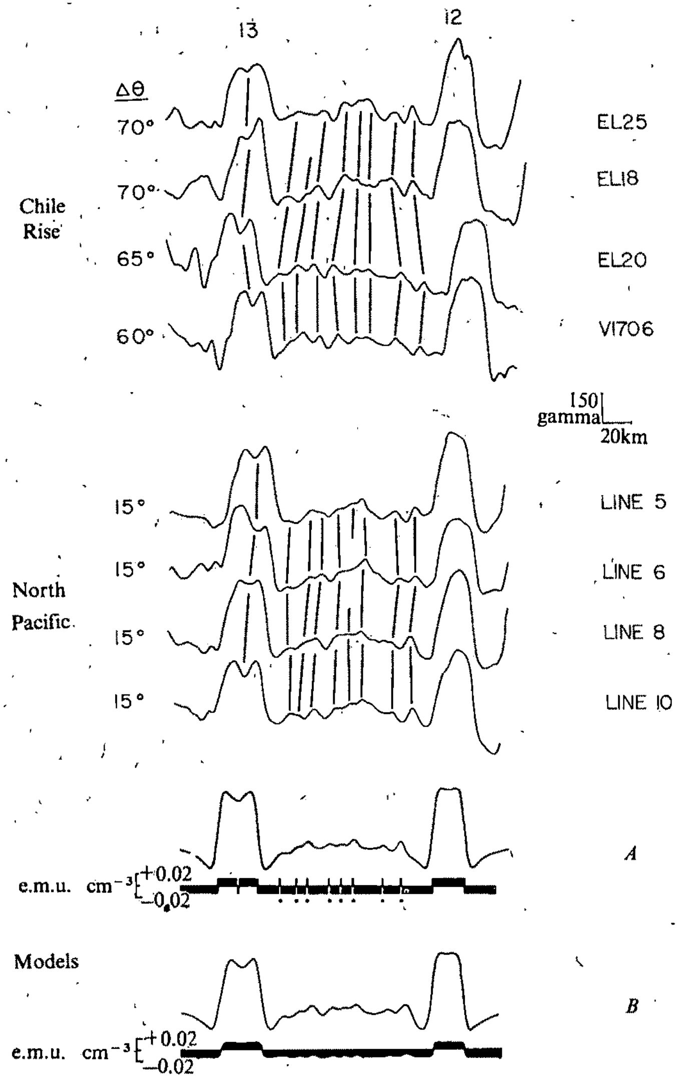

Fig. 2 Correlations of small scale features between anomalies 12 and 13. Models explained in text. Darkened figures represent magnetisation distributions. Dots in model *A* indicate locations of possible new events. Model *A* parameters: depth, 4.5 km; layer thickness, 0.5 km; smoothing (σ), 0.5 km; Δθ, phase shift parameter[3].

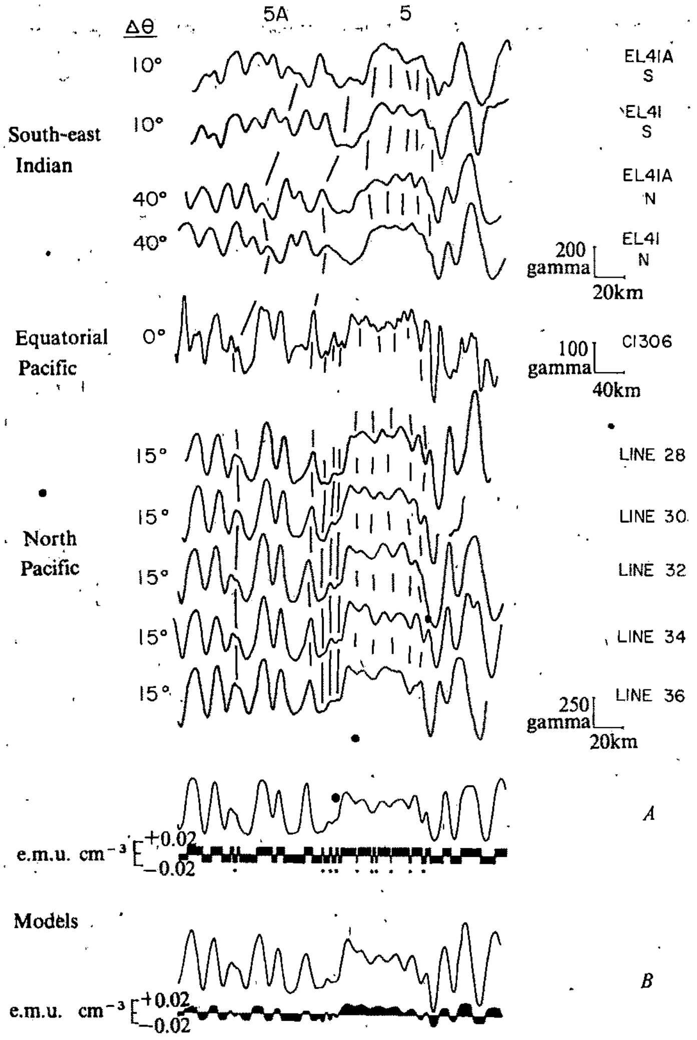

Fig. 3 Correlations of small scale features between anomalies 5 and 5A. Model parameters: depth, 3.5 km; layer thickness, 0.5 km; smoothing (σ), 0.5 km; Δθ, phase shift parameter[3].

Surface marine magnetic profiles lack sufficient detail to discriminate between a crustal magnetisation distribution due to short periods of reversed polarity and one due to intensity fluctuations in the palaeomagnetic field. Models *A* and *B* (Figs 2 and 3) demonstrate that both hypotheses can account for the observed small scale anomalies. Model *A* represents the 'reversal model' in which the field maintains constant intensity during periods of constant polarity. The duration of the polarity transition is assumed to be 5×10^3 yr (ref. 17). Model *B* represents the 'intensity fluctuation model' in which the intensity varies irregularly during periods of constant polarity. The models do not duplicate any one profile exactly. Instead they approximate the general character of all the profiles. The model magnetisation distributions have been smoothed with a Gaussian function to simulate the effect of such processes as dike injection and extrusion of lavas. The amount of smoothing is measured by the standard deviation, σ, of the Gaussian function[3,10]. Based on the results of near-bottom surveys[18,19] we assumed a standard deviation, σ, of 0.5 km.

Model *A* shows the possible location of short polarity reversals required to duplicate the observed anomaly pattern. These new events are almost all less than 3×10^4 yr in duration. We have inserted eight new events between anomalies 12 and 13 and 10 new events between anomalies 5 and 5A.

The existence of additional short period events in the time scale has been predicted by Cox[15] on the basis of a stochastic model. Assuming these features are due only to polarity reversals, the insertion of these new events changes the observed distribution of polarity interval lengths. Since these small scale features are seen throughout the time covered by the North Pacific survey area (40 m.y. BP to present), this suggests that the mean rate of polarity reversals may be considerably greater than previously estimated[2,15], perhaps by a factor of two. During intervals previously thought to be of constant polarity, the field may have behaved like a monostable oscillator, favouring one polarity more than the other. In particular, this behaviour may characterise the early Cainozoic where the Heirtzler *et al.* time scale identifies many long periods of constant polarity. Such monostable behaviour may be incompatible with a simple stochastic model like the Poisson process.

Model *B* demonstrates an alternative hypothesis that these small scale features may be due to intensity fluctuations within periods of constant polarity. The features were modelled by fluctuation with periods of 3×10^4 yr to 2×10^5 yr and amplitudes of 15% or more of the mean field intensity. The magnetisation shown in model *B* is not a unique representation of the actual field behaviour. The small scale features could be modelled by shorter period fluctuations of greater amplitude or the observed features may be artefacts of an even more complicated behaviour.

We considered the possibility that the intensity of fluctuations in model *B* might be explained by variations in the dipole moment of the field, variations in the intensity of the non-dipole field or variations in the pole position of the dipole field. The high coherence of the small scale anomalies recorded in different hemispheres over millions of years eliminates the possibility that they are due to variations in the non-dipole field. Variations in the pole position of the dipole field would have produced a phase difference of 180° between the variations observed in the Northern Hemisphere and Southern Hemisphere. This relative phase difference is not apparent in our profiles. Therefore, we conclude that the intensity fluctuations of model *B* would be due to variations in the dipole moment.

Near-bottom magnetometer profiles in areas where small scale lineated anomalies are observed at the surface may help to resolve the nature of the field behaviour responsible for the anomalies.

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North Sea Geothermal Gradients

REGIONAL studies of geothermal gradient patterns obtained from bottom hole temperature measurements in deep oil exploration boreholes have been the subject of several recent papers[1–3]. Harper[1] has produced approximate gradients in a study of the North Sea by the direct use of bottom hole temperatures routinely taken during the logging of exploration wells. These temperatures, which are taken with a maximum mercury-in-glass thermometer at a point typically 30 foot from the bottom of the hole, are, in general, less than the true formation temperature as a result of the cooling effect of the circulating drilling fluid.

The effect of such a thermal disturbance in a borehole may be approximated by a constant line heat source in a uniform infinite medium with a solution as a function of time, as derived by Lachenbruch and Brewer[4]. That solution, for a fixed depth in a borehole, is

$$T(t_2) = K\log(1+t_1/t_2) + T(\infty) \qquad (1)$$

where t_1 is the time of cooling the formation by the circulating fluid, t_2 is the interval from cessation of cooling to temperature measurement, $T(t_2)$ is the observed temperature at time t_2, $T(\infty)$ is the true formation temperature and K is a constant. Thus where multiple values of $T(t_2)$, t_1, and t_2 are known, equation (1) may be applied as a semi-log plot of $T(t_2)$ against $(1+t_1/t_2)$ to produce accurate formation temperatures at the

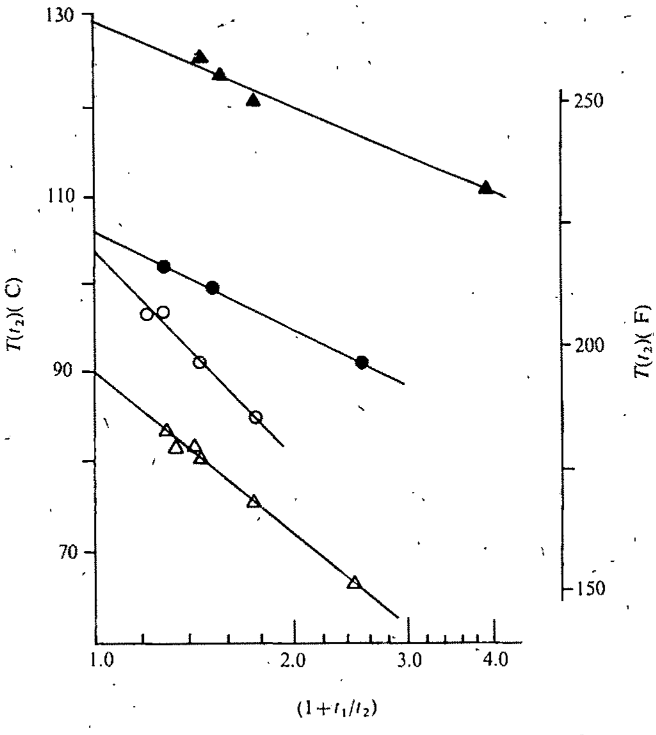

Fig. 1. Thermal recovery of four North Sea exploration boreholes. The temperature intercept is the true formation temperature. ▲, BP, UK; ●, Conoco, UK; ○, Amoco, UK; △, BP, UK.

linear axis intercept as was recently shown by Timko and Fertl[5].

Bottom hole temperatures from fifty-nine North Sea oil exploration wells, forty in the UK sector, fifteen in the Norwegian, and four in the Dutch, were studied and true formation temperatures calculated by the above method. Figure 1 is a semi-log plot for four typical wells. This method was also applied to a further forty-four results from the previous study by Harper[1]. These were additional data without t_1 values and it was, therefore, necessary to estimate this parameter statistically. The interval t_1, the total time of cooling, is the sum of the time taken to drill the final 30 foot of hole plus any additional time during which fluid circulates after drilling ceases. A log linear relation for drilling rate against depth was observed, while circulation time approximated a normal distribution. These results, based on data from forty-six North Sea wells, allow a satisfactory estimation of t_1 and provide a basis for the application of equation (1).

The determination of all geothermal gradients incorporated the subtraction of sea bottom temperatures from the true formation temperatures with all depths being calculated from sea bottom. Defant[6] showed that the bottom temperature of the central North Sea varies both with water depth and season of the year. Figure 2 shows the transformation of Defant's results to a mean annual temperature at sea bottom as a function of water depth. This result was combined with the true formation temperatures for the calculation of gradients. Geothermal gradients calculated from bottom hole temperatures uncorrected for the thermal disturbance of the drilling fluid would typically be lower than true gradients by 10–15%.

A geothermal gradient contour map, Fig. 3, is based on the total one hundred and three determinations with individual results being averaged over petroleum lease blocks which are 8 by 12 miles in the UK sector and somewhat larger in the others. As stated by Harper[1] and confirmed here, the regional trends seem to be governed by the major structural elements. The major sedimentary basins, English, SW Netherlands, German, Norwegian, East Shetlands, Moray Firth, and Midland Valley are all areas of high geothermal gradients with values locally reaching 44° C km^{-1}. Structural highs including Ringkobing-Fyn, Norwegian Platform, Mid North Sea, Mid Netherlands, and Grampian are characterised by low gradients which, south of Norway, approach 18° C km^{-1}. A geothermal high confirmed by four wells, located at approximately 55° N, 00° E, seems to confirm Armstrong's[7] opinion of the probable presence of Carboniferous coal-bearing rocks at a distance of one hundred miles from the Yorkshire coast trending ESE to fifty miles from the Norfolk coast. A thin stratum of coal above the bottom hole temperature measurement point with thickness 2–3% of that of the total formation would lower the overall thermal conductivity by approximately 25% due to its very low thermal conductivity[8], and could thus be a viable explanation of this anomaly. In addition there is a strong correlation, north of 56°, with the recently published Palaeocene isopach and base of Tertiary structure maps[9]. The coincidence of high geothermal gradients with the Tertiary depocentres reflects the lower thermal conductivity of this predominantly marine shale sequence.

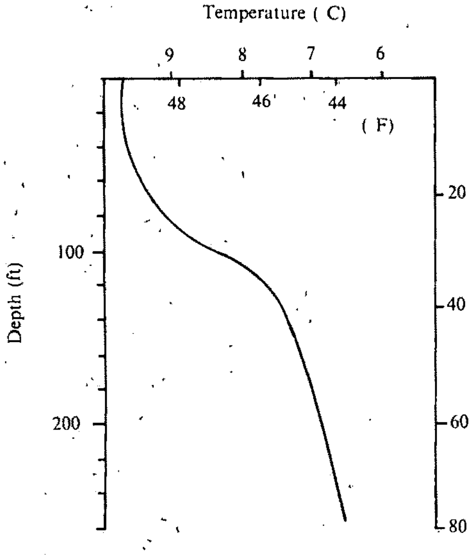

Fig. 2 Mean annual North Sea bottom temperature against water depth.

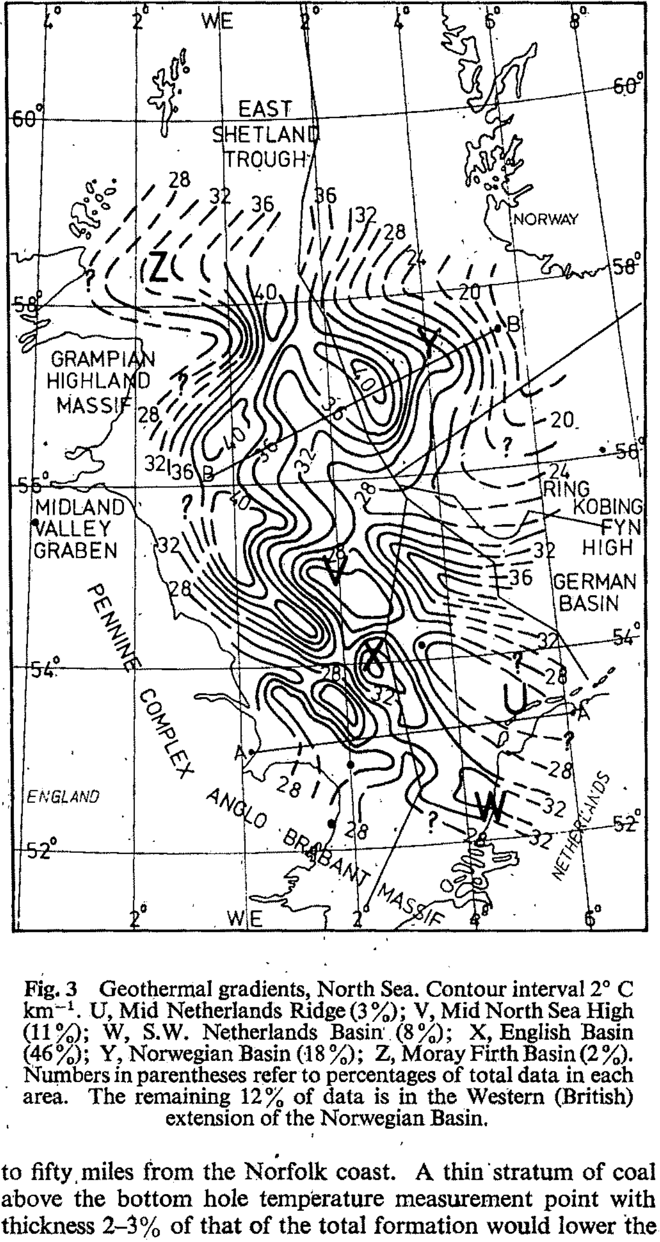

Fig. 3 Geothermal gradients, North Sea. Contour interval 2° C km^{-1}. U, Mid Netherlands Ridge (3%); V, Mid North Sea High (11%); W, S.W. Netherlands Basin (8%); X, English Basin (46%); Y, Norwegian Basin (18%); Z, Moray Firth Basin (2%). Numbers in parentheses refer to percentages of total data in each area. The remaining 12% of data is in the Western (British) extension of the Norwegian Basin.

Figure 4 incorporates diagrammatic cross sections of the

Table 1 Estimates of Thermal Conductivity

Section	Dominant lithology	Thermal conductivity [W m^{-1} K^{-1}]
Quaternary/Tertiary	Shale[11]	1.76 (ref. 3)
Cretaceous (including Danian)	Chalk[7,11]	1.84 (unpublished observation)
Jurassic	Sand/shale[10,11]	2.64 (ref. 3)
Triassic	Sand[7,10,11]	3.10 (ref. 3)
Upper Permian (Zechstein)	Evaporite/ carbonate[7,10,11]	3.85 (ref. 12)
Lower Permian (Rotliegendes)	Sand[10,11]	3.10 (ref. 3)
Carboniferous	Shale/ limestone[10,11]	1.94 (ref. 12)

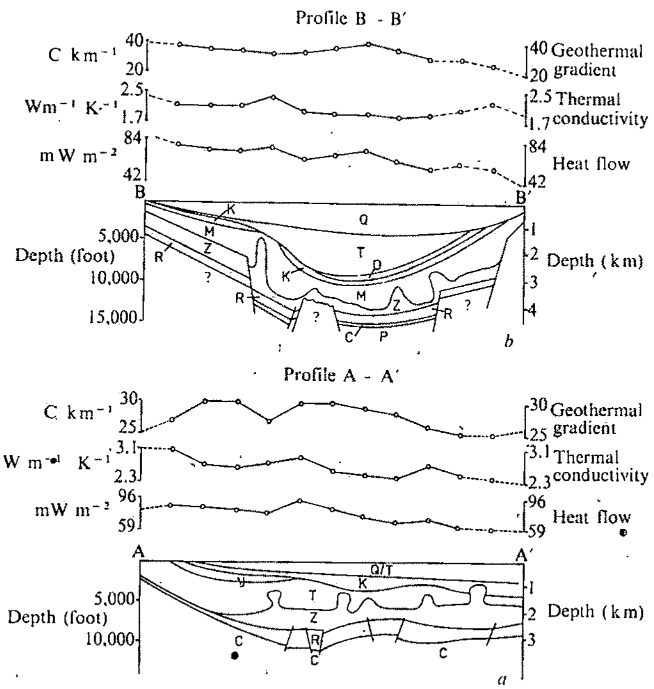

Fig. 4 *a*, Thermal and geological profiles of the southern North Sea. Geology after Armstrong[7]. Data points are samples from the contour map, Fig. 3, taken at equispaced points on profile A–A′. Q, Quaternary; T, Tertiary; D, Basal Tertiary/Upper Cretaceous (Danian); K, Cretaceous; J, Jurassic; T, Triassic; M, undifferentiated Jurassic/Triassic; Z, Upper Permian (Zechstein); R, Lower Permian (Rotliegendes); C, Carboniferous. *b*, As *a* but for central North Sea, profile B–B′.

North Sea in the positions indicated on Fig. 3 as well as the geothermal gradient, conductivity, and heat flow profiles. Estimates of thermal conductivity (Table 1) were made from published descriptions of the major lithological units of the North Sea together with the measured thermal conductivities of similar materials. Mean estimated heat flows of 75 mW m^{-2} from the southern profile (A-A′) and 71 mW m^{-2} from the northern profile (B-B′) were obtained, thus indicating no significant difference despite the higher gradients observed to the north. The values of heat flow, while slightly higher than the world mean[13], are intermediate in terms of other European basin values[1] and in good agreement with the world-wide mean for marginal seas[14]. A possibly significant aspect of Fig. 4 is that both heat flow profiles show an apparent decrease towards the European continent. This is consistent with world-wide heat flow means of 61.9 mW m^{-2} for post-Precambrian orogenic areas such as Great Britain and 38.5 mW m^{-2} for Precambrian shields such as Norway[14]. Seven recent combined heat-flow production measurements in Precambrian and Permian igneous rocks in southern Norway support such a value[15]. But, in general, the paucity of heat flow data in this area limits any attempt to confirm this trend.

Recent oil discoveries in the North Sea such as the Forties (57° 40′ N, 1° 0′ E) and Ekofisk (56° 30′ N, 3° 10′ E) fields seem to support the theories of Klemme[16] that high geothermal gradients enhance petroleum mobility, that is, migration to structural traps, to a greater degree than they adversely affect reservoir quality.

We thank the following oil companies for permission to use temperature data from their North Sea wells: Amoco, BP, Conoco and Phillips. We also thank Drs M. L. Harper and H. Y. Tammemagi for advice.

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Quantitative Tar and Plastic Waste Distributions in the Pacific Ocean

WE report in this letter the first quantitative data on tar and plastic waste distributions in the surface waters of the Pacific Ocean. During the Canadian Transpac-72 cruise, thirty-seven surface tows were made with a neuston net to collect particulate pollutants quantitatively. (The net, a Kahl Scientific model with a mouth 80 cm by 30.5 cm and a 150 μm mesh net, was towed at 4 to 5 knots for 10 to 15 min at a time, covering an average area of 1,800 m^2. It was rigged so that it would tow off to the side of the ship through water undisturbed by the ship's wake.) The research ship, CSS Parizeau (track shown in Fig. 1), left Tokyo on October 10, 1972, proceeded easy along latitude 35° N to near the California coast, then turned north along 125° W to reach Victoria, British Columbia, on November 2, 1972.

The tar distribution is shown in Fig. 2. Tar was present in thirty of thirty-three tows along 35° N as black or brownish-black lumps up to 3 cm in diameter. Its concentration was consistently higher in the Western Pacific from Tokyo to waters north of the Hawaiian Islands with a maximum of 14 mg m^{-2} wet weight and an average of 3.8 mg m^{-2}—the same order of magnitude as the weight of the organisms caught in the neuston net. The concentration of tar in the Eastern Pacific was an order of magnitude lower on average. No tar was found along 125° W. These tar aggregates were inhabited by diverse biological communities: diatoms and bryozoans, usually on the smaller pieces, and blue-green algae and goose barnacles on some of the larger ones. Crabs, mostly still in the megalops stage, and, as in the Atlantic[1–3], the isopod *Idotea metallica* seemed to be living on the lumps. Thus, the tar is apparently not directly toxic to many species, although incorporation of small lumps into the food chain by way of surface-feeding fish and copepods has been suggested as a hazard[1,4].

As shown in Fig. 2, plastic is widespread, although in minute quantities. It was present in twenty-one of thirty-three tows along 35° N as small (0.1 to 0.5 cm), round, colourless pellets weighing 20 to 50 mg each. In contrast to tar, the maximum concentration of plastic was found in the Eastern Pacific (3.5 mg m^{-2}). The average concentration was 0.3 mg m^{-2}. The maximum number of pieces observed was sixty-two, corresponding to 34,000 pieces km^{-2}. None was found along 125° W. Smaller plastic pieces did not appear to support

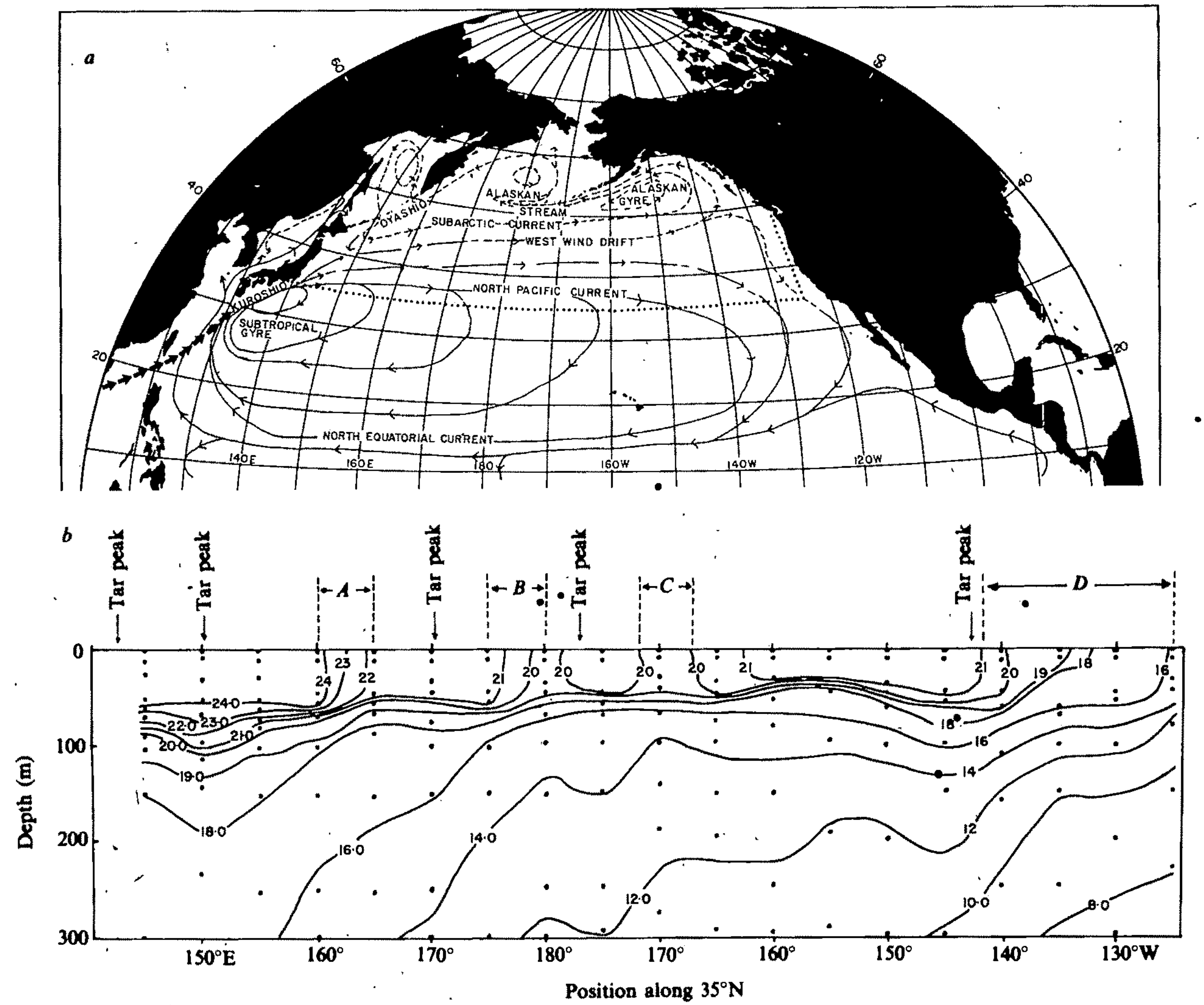

Fig. 1 *a*, Track of the CSS Parizeau on Transpac-72 cruise (···), the surface transport in the North Pacific Ocean[9] (cold currents, - - - ; warm currents, ——), and the Persian Gulf–Japan tanker route (heavy arrows). *b*, Temperature distribution (° C) in the surface waters along 35° N in the Pacific Ocean. The zones of influx of cold subarctic water (*A*, *B*, *C*, *D*) and the locations of the peak concentrations of tar are indicated.

a fouling community, although larger ones had growths similar to that on tar lumps of the same size.

Some paper, elastic bands and wood were present in the first few tows off Japan (up to 500 miles from Tokyo). None was found, however, in the remaining 6,000 mile journey.

The persistent tar distribution may result from several factors: likely sources from marine oil transport, augmented by accidental spills, prevailing wind transport and surface circulation.

First, an order of magnitude difference in 'possible-source' volumes of oil shipments to Japan and the west coast of the United States correlates well with a similar order of magnitude difference in tar for Western and Eastern Pacific waters, assuming that persistent tar originates mainly from tanker washings. In the North-western Pacific, 6,000 t.b.d. (thousand barrels per day) of crude oil were transported by tankers in 1971, 90% going to Japan along the Persian Gulf–Japan tanker route (Fig. 1). In the North-eastern Pacific, only 600 t.b.d. of seaborne crude oil were handled. The locations of the tanker routes do not, however, agree with the detailed distribution pattern of pelagic tar. (Information on oil tanker routes is from ref. 5.)

Second, prevailing winds are most effective in transporting surface pollutants. In general, a high concentration of tar in the Western Pacific between 150° E and 175° W and in the Eastern Pacific at 143° W is consistent with the mean annual wind stress over the North Pacific[6]. The wind stress tends to move surface pollutants eastwards in the Western Pacific to about 170° W where both a change to a southward direction and a weakening in wind stress takes place. The 143° W peaks of tar and plastic are in a region of zero annual wind stress, where accumulation of both tar and plastic is expected. This does not explain the absence of plastic in the Western Pacific, which possibly reflects the liberal input from the Hawaiian Islands relative to Japan.

Third, surface circulation also affects the long term distribution of surface pollutants. The cruise track of Transpac-72 at 35° N crosses an area influenced by both warm subtropical water and colder subarctic water. The fast moving warm Kuroshio Extension current flows east from 36° N, 140° E, in a remarkable meandering path up to about 160° E, where it transforms into the weak, broad North Pacific Current[7]. The waters further east undergo many seasonal and spatial variations due to mixing and incursion of cold subarctic water across the normal boundaries of the warm subtropical gyre water[8]. East of 140° W, the water is mainly of subarctic origin. The probable paths of intrusion of subarctic water can be inferred from a summary of North Pacific transport[9] and from the temperature distribution, both shown in Fig. 1: (*A*) 160° E to 165° E at about 22° C; (*B*) 175° E to 180° W with temperature slightly below 20° C; (*C*) 173° W to 167° W at about 19.5° C; (*D*) east of 142° W with very rapid drop in temperature from 21° C to 16° C at 125° W. (Figure 1 was modified to include recent data on the North Pacific circulation supplied

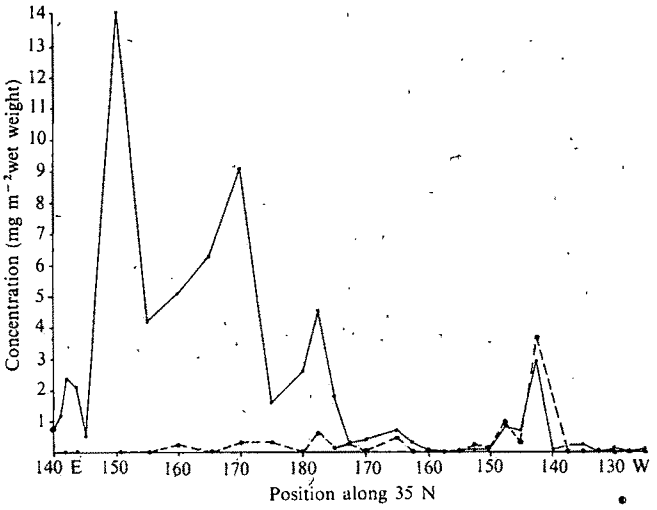

Fig. 2 Distribution of tar (——) and plastics (- - -) along 35° N in the Pacific Ocean. Tows covered an area of 1,800±300 m^2.

by R. E. Thomson.) The tar peaks at 150° E, 170° E, 178° W and 143° W seem to be in the slack, warmer subtropical water separated by the above zones of colder subarctic water, which have a low tar content. It is worthwhile to note that the time series at ocean weather station 'Papa' (145° W, 50° N) in subarctic water shows zero tar values (unpublished data of Ocean Chemistry Division, Marine Sciences Directorate, Pacific Region, Department of the Environment, Victoria, BC, Canada). Our observations are also in agreement with known features in the Atlantic[10] which show much higher concentrations of tar in the subtropical Sargasso Sea.

Due to the highly complex interaction of these three factors in the ocean, we cannot explain the waste distribution satisfactorily from one set of data. Our values in the Pacific, combined with those obtained in portions of the Atlantic and in the Mediterranean by other workers[1,2,10,11], do, however, indicate that the tar and plastic wastes are widespread on the surface of the oceans.

We thank William Heath for biological identifications, the captain and crew of the CSS Parizeau for help with the sampling and Drs R. W. Stewart and R. E. Thomson of Marine Sciences Directorate, Pacific Region, for criticism.

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BIOLOGICAL SCIENCES

Replication of Yeast Chromosomal DNA

THE study of replication in higher eukaryotes has been hampered by the large size of the DNA molecules in their chromosomes. The yeast *Saccharomyces cerevisiae* is a eukaryote with very little DNA per haploid nucleus (9×10^9 daltons)[1]. This has facilitated measurement of intact yeast chromosomal DNA by sedimentation velocity[2] and electron microscopy[3]. The results indicate that yeast chromosomes are similar in size to those of viruses and bacteria, each chromosome containing a single DNA duplex ranging from 10^8 to 10^9 daltons. More than 95% of the chromosomal DNA molecules isolated from asynchronous cultures are simple linear structures, but the few branched structures are good candidates for replication intermediates[3]. We have used two experimental approaches which provide evidence that these exceptional molecules are, indeed, replication intermediates. Characterisation of these

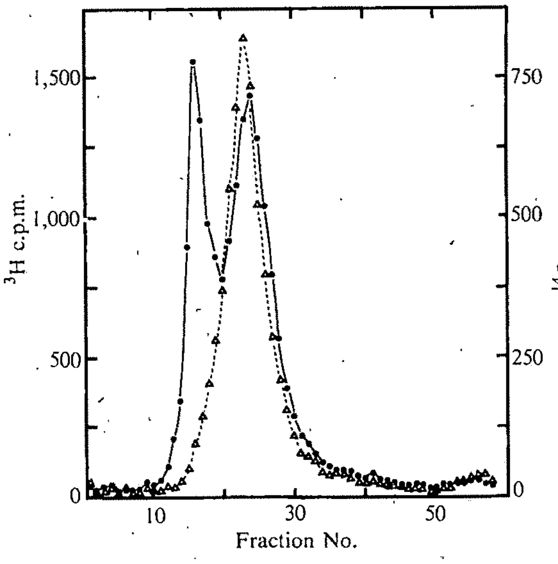

Fig. 1 Density profile of yeast DNA from a culture shifted from dense to light medium. A derivative of A364A D5 (ref. 13), A364A D5 U^+A^+, which does not require uracil and adenine was grown for many generations at 30° C in ^{15}N-D_2O medium containing 0.25 µCi ml^{-1} 2-^{14}C-uracil (New England Nuclear Corp., 0.5 µCi μg^{-1}). The medium contains (per litre) 1.45 g Difco yeast nitrogen base (without amino acids or $(NH_4)_2SO_4$), 10 g succinic acid, 6 g NaOH, 50 mg tyrosine, 20 g glucose, 100 mg $^{15}NH_4Cl$ (Isomet, Oakland, New Jersey) and 1 litre D_2O (Bio-Rad Laboratories, Richmond, California). The cells were filtered on Millipore HA filters and resuspended in Y-minimal medium (6.79 g Difco yeast nitrogen base (without amino acids), 10 g succinic acid, 6 g NaOH l^{-1}) supplemented with 20 g glucose and 50 mg tyrosine l^{-1} and containing 10 µCi ml^{-1} 6-^{3}H-uracil (New England Nuclear Corp., 250 µCi μg^{-1}). After incubation for 100 min, cells were collected by centrifugation and spheroplasted as described previously[2] at a concentration of 10^8 cells ml^{-1}. Spheroplasts were washed by centrifugation at 4° C with a solution containing 1 M sorbitol, 0.1 M sodium citrate and 0.01 M Na EDTA, *p*H 5.8, suspended in the same solution, and 0.3 ml (about 6×10^7 spheroplasts) was layered over 0.1 ml of 10 mg ml^{-1} pronase (previously treated at 70° C for 10 min) on top of 7 ml CsCl (Harshaw Chemical Co., Solon, Ohio) solution ($\eta=1.4040$) containing 0.01 M Tris-Cl (*p*H 8) and 0.01 M Na-EDTA (*p*H 8) in a centrifuge tube. The spheroplasts were lysed by the addition of 0.1 ml 10% sarkosyl. After 30 min at room temperature, gradients were overlayered with mineral oil and centrifuged in a Spinco 50 Ti rotor at 20° C and 33,000 r.p.m. for 60 h. Gradients were collected from the bottom of the tube through a 13 gauge needle, which had been boiled in 0.1 M EDTA, at a rate of less than 0.5 ml min^{-1}. DNA radioactivity was measured on 50 µl portions of gradient fractions as described previously[2]. ●, ^{14}C; △, ^{3}H.

molecules by electron microscopy indicates that yeast DNA replication is similar to higher eukaryotic DNA replication in two important respects. First, most and possibly all initiations are internal. Second, many chromosomes contain more than one replication unit.

Material enriched in replicating structures can be obtained by transferring cells from isotopically dense to isotopically light medium and banding the DNA in a density gradient. Partially replicated molecules have buoyant densities intermediate between unreplicated and completely replicated molecules. Figure 1 shows the buoyant density profile of yeast DNA prepared from a culture grown in dense medium and transferred to isotopically light medium 100 min before collection. The peak containing only ^{14}C radioactivity corresponds to fully dense DNA and the peak containing both ^{3}H and ^{14}C radioactivity contains hybrid density DNA. When DNA from the region between the peaks (fractions 19, 20 and 21) was examined in the electron microscope 31% (thirty out of ninety-seven) of the molecules seen contained either Y-shaped terminal forks or internal 'bubbles' (Fig. 2). These bubbles are similar to structures observed in λ (refs 4 and 5), T4 (ref. 6) or T7 (ref. 7) replicating DNA which result from pairs of diverging replication forks arising from single initiation sites. In molecules which were Y-shaped, two of the arms were equal in length. In the case of bubbles, the two branches between the forks were equal in length. These structures must be almost entirely composed of double stranded DNA because under the spreading conditions used single stranded DNA collapses on itself in 'bushes'[8]. However, small single-strand regions of the sort found in bacteriophage λ (ref. 10), T4 (ref. 6) and T7 (ref. 11) replication structures might not have been detected.

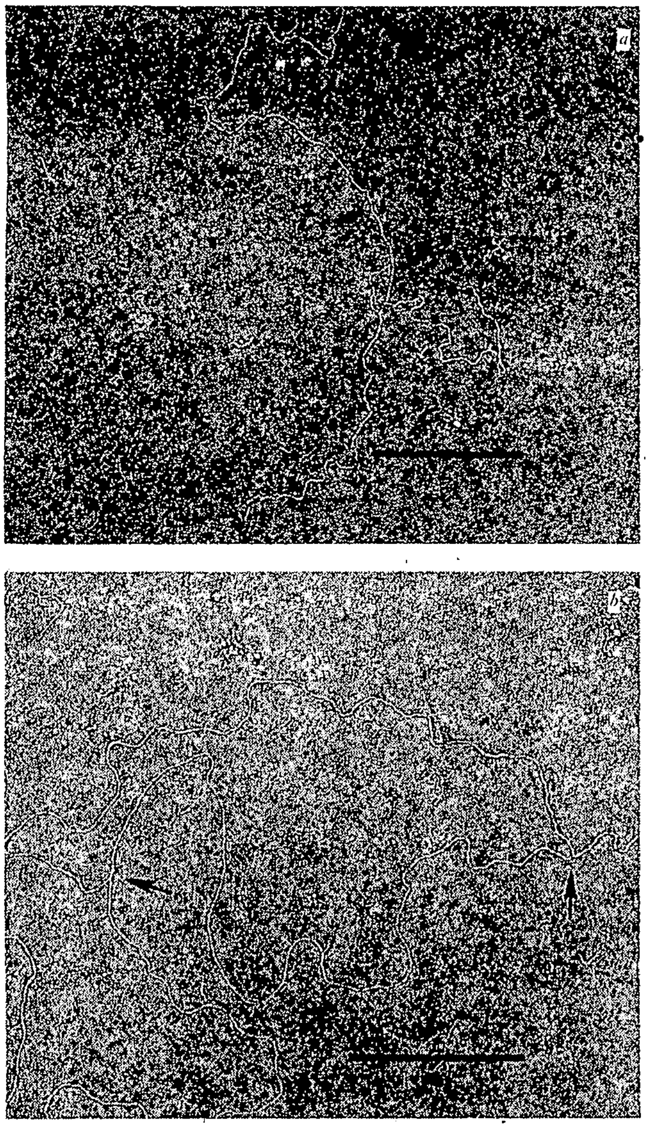

Fig. 2 Electron micrographs of portions of yeast DNA replication intermediates. DNA from CsCl gradients (Fig. 1) or sucrose gradients (Fig. 3) was spread using a modification of the aqueous procedure of Davis, Simon and Davidson[8] which has been described in detail[3]. Molecules were photographed using a Philips EM 300 electron microscope. Contour lengths in cm were determined using a Keuffel and Esser map measurer and were converted to microns by comparing the contour lengths of yeast DNA to the contour length of intact bacteriophage T4 DNA[3] spread under the same conditions. T4 DNA has a well characterized molecular weight (1.2×10^8) and contour length (52 µm)[9]. *a*, A Y-shaped fork with arms 2.2 µm long. *b*, A bubble with branches between the forks 5.5 µm long. The bars represent 1 µm. The arrows indicate forks.

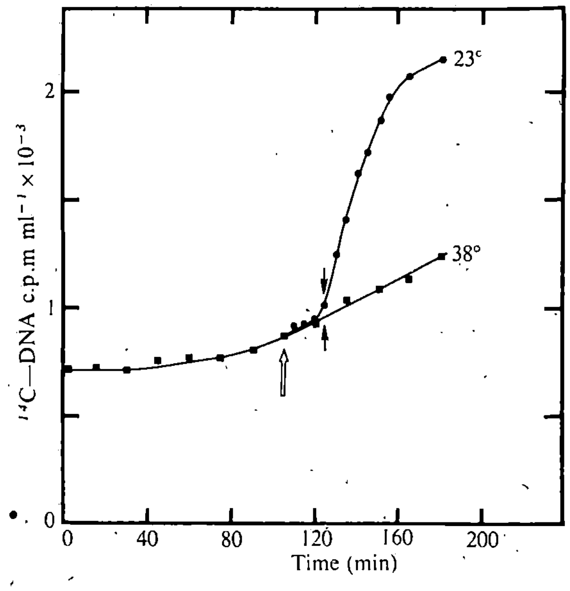

Fig. 3 Pattern of DNA synthesis in a synchronous culture. A culture of the temperature-sensitive cell division cycle mutant *cdc* 7 (strain 4008, obtained from L. H. Hartwell) was grown at 23° C in Y-minimal medium supplemented with 20 g glucose, 0.5 g yeast extract, 20 mg uracil, 20 mg adenine, 50 mg tyrosine, 50 mg histidine, and 50 mg lysine per litre and 1 µCi ml^{-1} 2-^{14}C-uracil (24 mCi mmol^{-1}, Schwarz/Mann) to a density of 2.5×10^6 cells ml^{-1}. To avoid holding cells at 38° C for a long time, the culture was synchronised by treatment with α-factor for one generation (3.5 h) as described previously[12]. To remove α factor, cells were filtered on Millipore HA filters, washed with 200 to 400 ml medium at 38° C and resuspended in the original volume of medium at 38° C ($t=0$). After 105 min at 38° C (arrow), the culture was divided and half was shifted to 23° C. Twenty minutes later samples were taken from each culture (small arrows) and DNA was prepared for electron microscopy by sedimenting it away from smaller cellular components on sucrose gradients as described previously[3]. Incorporation of ^{14}C-uracil into DNA was determined as described previously[13].

If these structures are associated with replication, samples from the fully dense and fully hybrid peaks should contain mostly simple linear molecules. In DNA from fraction 16 (Fig. 1), the peak of the fully dense region, seventy-nine out of eighty molecules examined were simple linear structures.

Replication intermediates should be found in cells in S phase, but not in cells at other stages in the cell cycle. To examine this we used a temperature-sensitive mutant defective in the initiation of DNA synthesis. In a culture of this mutant maintained at the non-permissive temperature (38° C), cells accumulate in the cell cycle just before DNA synthesis (unpublished results of L. M. H. and L. H. Hartwell). Figure 3 shows that when such a culture is shifted to the permissive temperature (23° C), it undergoes a synchronous round of DNA synthesis. The DNA synthesised in the culture maintained at 38° C is primarily mitochondrial DNA (unpublished results of C. S. N.). Twenty minutes after the temperature

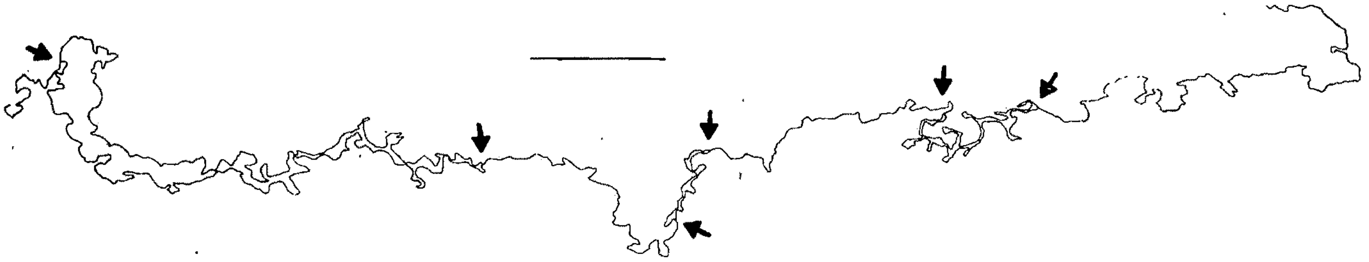

Fig. 4 Tracing of an electron micrograph of a replicating molecule. DNA was prepared and photographed as described in Fig. 2. The molecule shown is 117 μm long and the bubbles (right to left) are 13, 6, and 35 μm. Branch points are indicated by arrows. The bar represents 5 μm.

shift, samples were taken from the culture maintained at 38° C and the culture which had been shifted to 23° C (arrows, Fig. 3) and DNA was prepared for electron microscopy.

In the DNA from the sample collected at 38° C, 149 out of 150 molecules examined were simple linear structures. In the sample collected 20 min after the shift to 23° C, 66% (thirty-nine out of fifty-nine) of the DNA molecules observed contained bubbles or forks of the kind found in the density experiments. The correlation of such molecules, which are not present in the mutant at the restrictive temperature, with the burst of DNA synthesis, as measured by incorporation of radioactive label, is again consistent with the hypothesis that these molecules are replication intermediates. The total amount of DNA replicated in this sample can be estimated from the ratio of the sum of lengths of one arm of all bubbles and forks to the total length of all molecules measured. This ratio is 0.1 and is consistent with the incorporation data (Fig. 3), indicating that about 6% of a round of replication has occurred at the 20 min time point. The replicating molecules isolated from the cells in S phase varied in size from 47 to 250 μm. Thus, they probably represent intact chromosomal DNAs[3], and it is unlikely that extensive double-strand breakage occurs during the isolation of the molecules or during yeast replication.

From analysis of the data obtained in the synchronous culture experiment, a number of general conclusions can be made about yeast chromosomal DNA replication. The appearance of bubbles is most easily explained by internal initiation. Since most (thirty-four out of thirty-nine) of the replicating molecules contained bubbles, it is likely that most yeast DNA molecules initiate synthesis internally. The presence of Y-shaped molecules suggests either that there are some initiation sites at the ends of the chromosomes, or that internal initiations occur sufficiently close to the ends of some chromosomes that, after a small amount of DNA synthesis, a Y-shaped intermediate is formed. Bacteriophage T7 DNA has a Y-shaped intermediate throughout most of its replication although it initiates internally[7].

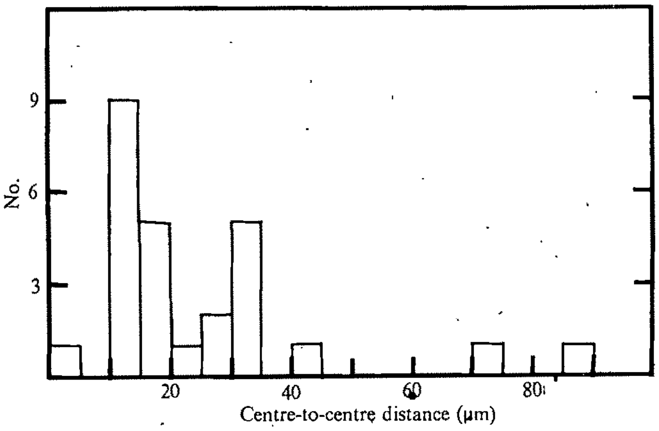

Fig. 5 Distribution of centre-to-centre distances between bubbles in molecules containing more than one bubble. Measurements of the centre-to-centre distances between bubbles in the replicating molecules from the experiment in Fig. 3 were made with a Keuffel and Esser map measurer on photographs at a magnification of 10,000.

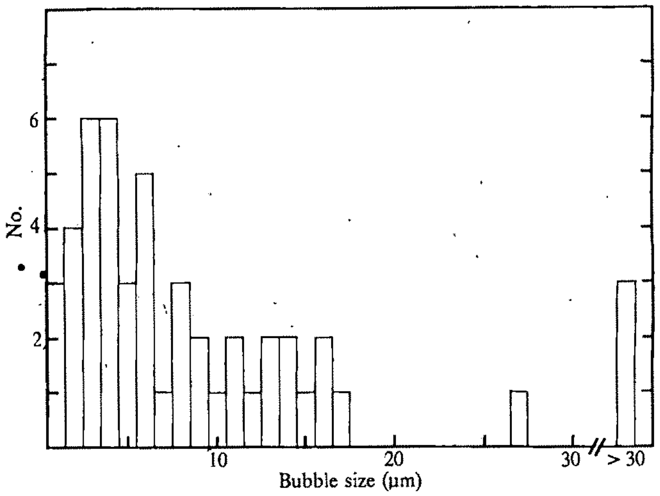

Fig. 6 Distribution of bubble size. Measurements of the size of bubbles in replicating molecules from the experiment in Fig. 3 were made on photographs at a magnification of 10,000 to 23,000.

At least some yeast chromosomes contain multiple initiation sites. In the S phase sample, 31% (twelve out of thirty-nine) of the replicating molecules contained more than one bubble. Figure 4 shows a tracing of a molecule with three bubbles. The size of replication units can be estimated from the spacing of bubbles along molecules. If a replication unit is defined as the amount of DNA replicated from a single initiation site, then a measure of the centre-to-centre distance between bubbles provides an estimate of this size[14]. The centre-to-centre distance between bubbles varied from 3 μm to 86 μm. Figure 5 shows that the distribution is not random; most of the distances fall into two clusters, one at 15 to 20 μm and the other at 30 to 35 μm. These values represent upper limits for replication unit sizes since some units may not have been activated in the observed molecules and some adjacent units may have already fused. Most bubbles are smaller than the smallest replication unit (Figs 5 and 6), so it is unlikely that many bubbles represent fused units. Thus it seems that replication unit size in yeast is more like that of higher eukaryotes[14,15] than that of prokaryotes[16,17].

The replication units on a chromosomal DNA molecule seem to initiate replication at different times. If all units initiated at the same time, then all bubbles on a molecule should be the same size (assuming that the rate of replication at all forks is constant); however, we find bubbles of varying sizes on individual molecules (for example, Fig. 4). The data also indicate that many yeast chromosomes in the synchronised cells initiate replication early in the S phase. This is shown by the fact that 65% of the molecules contained replication structures at a time when only about 10% of the DNA had replicated. Thus, at least 65% of yeast chromosomes initiate within the first 10% of S. This estimate could be in error for two reasons. Any asynchrony in the initiation of DNA synthesis among cells in the population would lead to an underestimate of the number of molecules which initiate synthesis at the beginning of S. In addition, mechanical shear during spreading of DNA could alter the apparent percentage of replicating molecules.

In summary, we find that yeast DNA replication intermediates include bubble-containing structures and Y-shaped structures. DNA molecules containing these structures are found in cultures in S phase, but not in cultures blocked before the initiation of DNA synthesis. In addition, there is at least a thirty-fold enrichment for molecules containing these structures in the region of a CsCl gradient where partially replicated molecules are expected to band. The same kinds of structures have been observed in DNA from asynchronous cultures[3]. Therefore, these structures cannot be attributed to abnormal growth conditions induced by density labelling or to the synchronisation procedure.

DNA replication in yeast is similar to replication in higher eukaryotes in several ways. Most, if not all, initiation sites are internal. Many chromosomes contain more than one replication unit, and the size of replication units is similar to that in higher eukaryotes. Because we can observe molecules which are almost certainly intact chromosomal DNAs, however, experiments which are very difficult to do in higher eukaryotes are possible. For example, replication maps of individual chromosomes can be made which include the location of initiation sites and the sequence of replication of different units.

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Preferential DNA Repair in Human Cells

EXCISION repair plays a vital role in the recovery of human cells from ultraviolet irradiation[1], but it does not remove all lesions from DNA, even when they are as potent as cyclobutane pyrimidine dimers. In fact, only about 50% to 75% of the dimers produced by low fluences of ultraviolet light are excised and the remainder persist in the DNA for at least 24 to 48 h[2–4]. We have investigated why some but not all dimers are excised. Our conclusion is that dimers, and possibly other ultraviolet photoproducts, persist in tracts of DNA which are rendered refractory to excision repair by a 'mask' of protein.

Human fibroblasts (WI-38) were grown in culture, labelled with ^{3}H-thymidine, irradiated with ultraviolet light, incubated for various periods and then enzymatically assayed[5] for DNA damage by using a *Micrococcus luteus* extract containing repair endonucleases (see legend to Fig. 1). This assay mimics the first step of *in vivo* excision repair, namely the endonucleolytic nicking of DNA in the vicinity of pyrimidine dimers, so we expected that, if nucleoproteins did indeed interfere with excision repair *in vivo*, they would likewise interfere with the *in vitro* assay for DNA damage. To test this hypothesis the cellular DNA was assayed for damage after exposure to either low (less than 0.15 M) or high (2 M NaCl) concentrations of salt, that is, conditions which ensured that histones, and possibly other proteins, either remained bound to, or were stripped from[6], the chromosomal DNA. We did in fact find that exposing the chromosomal DNA to a high concentration of salt greatly increased the

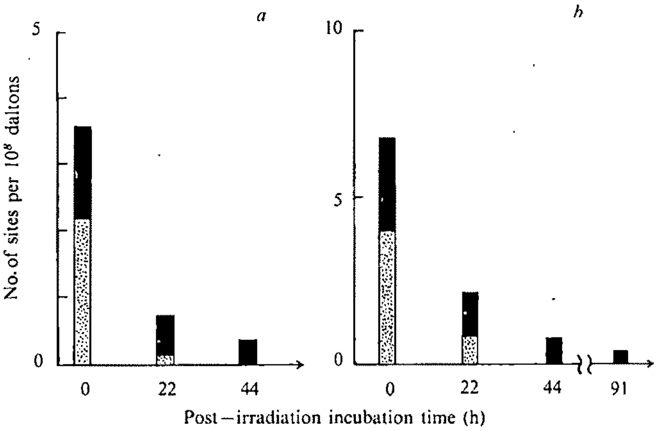

Fig. 1 Disappearance of ultraviolet-induced endonuclease sensitive sites from the DNA of human fibroblasts after 50 (*a*) or 100 (*b*) erg mm^{-2} of 254 nm ultraviolet light. The sites exposed under low salt conditions (dotted) and the additional sites exposed by high salt (black) were assayed essentially by the method described elsewhere[5]. Approximately 100,000 cells (WI-38 originally derived from embryonic lung tissue) were plated into 60-mm plastic dishes and incubated overnight in Eagle's medium with 10% dialysed foetal calf serum and 1 µCi of ^{3}H-thymidine added to label the DNA. After ultraviolet irradiation, cells were re-incubated for various times and then harvested by scraping into ice-cold saline-EDTA solution. For the endonuclease assay the cells were first rendered porous by osmotic shock, that is, they were concentrated to 5×10^6 cells ml^{-1} in 10% sucrose, then diluted six-fold in water and vortexed. The sample was then divided into two, one of which was made 2 M with respect to NaCl and kept at 0° C for 5 min. Both samples were then diluted a further ten-fold with water and then incubated with *M. luteus* extract for 12 min at 37° C. Fifty microlitres of each sample was then layered on an alkaline sucrose gradient so that the cellular DNA content could be sedimented through alkali to detect single-strand breaks produced by endonuclease action, that is, endonuclease-sensitive sites[5]. In control experiments using either unirradiated cells or no *M. luteus* extract, the weight average molecular weight of the DNA was always in the range of 0.9 to 1.1×10^8 daltons, so we normalised all our results to the number of endonuclease-sensitive sites per 10^8 daltons of DNA. These values are accurate to within ±0.1 site.

yield of detectable endonuclease-sensitive sites (Fig. 1). In cells which were assayed immediately after ultraviolet irradiation and had, therefore, no chance to repair DNA damage, over 60% more sites were revealed by the high salt assay. In other words, at least 40% of the chromosomal DNA is normally inaccessible to exogenous repair endonucleases used in the *in vitro* assay. This is a conservative estimate because some reassociation of protein and DNA probably occurs when the 2 M NaCl is diluted ten-fold to perform the assay. We do not believe, however, that much more than 50% of the DNA is inaccessible because in earlier experiments[5] employing low salt concentrations it was estimated that about half of the dimers induced in the DNA did give rise to detectable endonuclease sites.

The sites which are normally inaccessible to the exogenous repair endonucleases also seem to be relatively inaccessible to the DNA repair systems which act *in vivo*. Thus, while all of the ultraviolet-induced sites initially detectable under low salt assay conditions are removed from the DNA of cells during 44 h of post-irradiation incubation (Fig. 1 and ref. 5), many of the additional sites exposed by the high salt assay still persist in the DNA. For instance, 44 h after exposure to a fluence of 100 erg mm^{-2} of ultraviolet light, 28% of these additional sites still remain in the DNA and, even after 91 h, 13% still persist (Fig. 1).

The simplest explanation for our results is that some tracts of DNA are masked from the action of repair enzymes by sheaths of nucleoprotein. *In vitro* this protein can be removed from the DNA by high salt concentrations while *in vivo* it seems that repair systems can slowly gain access to the DNA, possibly because of changes that occur in DNA-protein complexes during DNA replication, or cell division, or both. We estimate that about 40% of the DNA is masked in this way and that the tracts covered with protein are no more than 10^8 daltons long because when using the low salt assay we did not observe any higher molecular weight DNA which was free of endonuclease-sensitive sites. Such conclusions are reasonable because it is known that about half of the DNA in the chromatin of mammalian cells is 'open' and linked together by many short tracts of DNA covered with protein[6].

Two general comments on our results are appropriate. First, it is possible that damage induced by agents other than ultraviolet light could also persist in these masked tracts of DNA and, as mammals age, their cells might accumulate such damage, especially in the absence of cell division. Second, differentiated cells might tolerate considerable damage in these masked, and possibly genetically unexpressed[7], portions of DNA. Thus the de- or re-differentiation of a cell might have a lethal, mutagenic or possibly carcinogenic[8] consequence if expression of this DNA occurred before repair could take place.

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Note added in proof. Recent work[9,10] using a similar technique also indicates that there are both fast and slow components to the repair system which removes ultraviolet-induced endonuclease sensitive sites from the DNA of human cells.

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Histone mRNA and Histone Synthesis during Embryogenesis

PROPORTIONAL contributions of individual histone fractions to total histone and their degree of microheterogeneity change during development. This has now been established for many plants and animals[1,2] including sea urchins[3-6]. The very lysine-rich histones, designated F1, can be fractionated as several proteins of similar amino acid composition[7,9] and as different phosphorylated derivatives[10,11]. These histones in particular show considerable species specificity in primary structure, molecular weight, and microheterogeneity[8,9,12,13]. It has been demonstrated in earlier studies[3-6] that aside from changes in proportion among histone classes synthesised during sea urchin development, synthesis of an F1 characteristic of the morula stage (F1-m) gives way to synthesis of a new and electrophoretically distinct F1 at the gastrula stage (F1-g). Significantly, the F1-m histone is neither degraded nor converted to F1-g during the interval from morula to gastrula[6].

We report here experiments designed to distinguish between two alternative modes of control over these developmentally programmed changes: differences in the template RNA from which histones are translated and

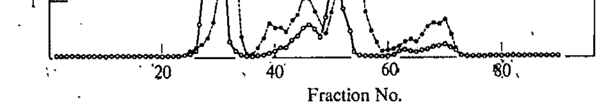

Fig. 1 Acetic acid-urea acrylamide gel electrophoresis of *L. pictus* embryo histones labelled *in vivo*. Two millilitres of morulae (approximately 2×10^6 embryos at about 100 cells) was incubated in 100 ml sterile seawater with 200 µCi ^{3}H-lysine (20.8 Ci mmol^{-1}) for 1 h. Two millilitres of gastrulae (0.5×10^3 to 1.0×10^3 cells) was incubated in a volume of 100 ml with 20 µCi ^{14}C-lysine (260 mCi mmol^{-1}) for 2 h. Histones were isolated[21] and dissolved in 2.5 M urea, 0.9 N acetic acid, 0.2% 2-mercaptoethanol. Electrophoresis was at 2 mA per gel for 5–6 h on 10% acrylamide, 2.5 M urea, *p*H 2.7 acetic acid gels, 14 cm long[18]. The histone-containing portion of the gel was sliced and hydrolysed for counting. ○—○, ^{3}H-lysine morula histones; ● - - - ●, ^{14}C-lysine gastrula histones.

differences in the way histones from an invariant soluble pool associate with DNA in the chromatin. The experiments are designed also to distinguish between primary structure differences and differential modification after synthesis as the chemical basis for the different electrophoretic mobilities of F1-m and F1-g.

Newly synthesised histones of *Lytechinus pictus* morulae and gastrulae were first examined by acetic acid-urea gel electrophoresis of the proteins labelled in a pulse exposure to ^{3}H-lysine[14]. The results are shown in Fig. 1. Morulae synthesise a lysine-rich histone (F1-m) that migrates more slowly in this electrophoretic system than does the F1 made by gastrulae (F1-g). Proportional differences of incorporation among the other four histone classes, particularly in the ratio F2b/F2a2, were observed as usual[6].

Morula and gastrula histones were then separated by electrophoresis on sodium dodecyl sulphate (SDS) gels, according to the method of Laemmle[15]. This analysis shows (Fig. 2) that morulae synthesise only F1-m, with molecular weight ≃20,000. First observed between hatching and gastrulation is the synthesis of F1-g (molecular weight ≃21,000). By the time gastrulation is in progress, incorporation of tracer amino acid into F1-m is greatly reduced and labelled F1-g accounts for most of the new F1. Electrophoretic mobilities of F1-m and F1-g are not altered detectably in either separation system after treatment with alkaline phosphatase (data not shown here). Seale and Aronson[4] report similar findings in experiments with another species, *Strongylocentrotus purpuratus*.

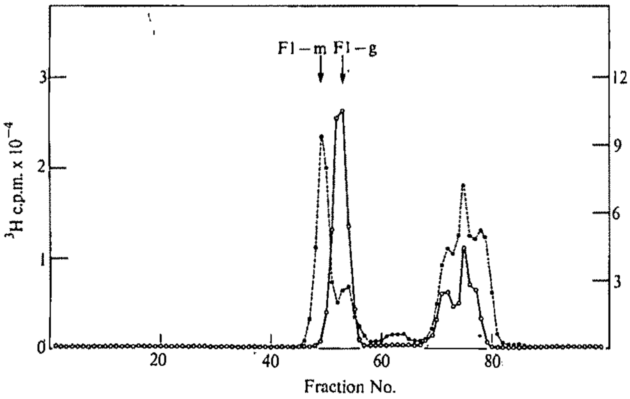

Fig. 2 SDS acrylamide gel electrophoresis of morula and gastrula histones. Histones, prepared as described under Fig. 1, were dialysed against 0.1% SDS, 0.2% 2-mercaptoethanol, and separated on 12.5% acrylamide-SDS gels[19]. ○—○, ^{3}H-lysine morula histones; ● - - - ●, ^{14}C-lysine gastrula histones.

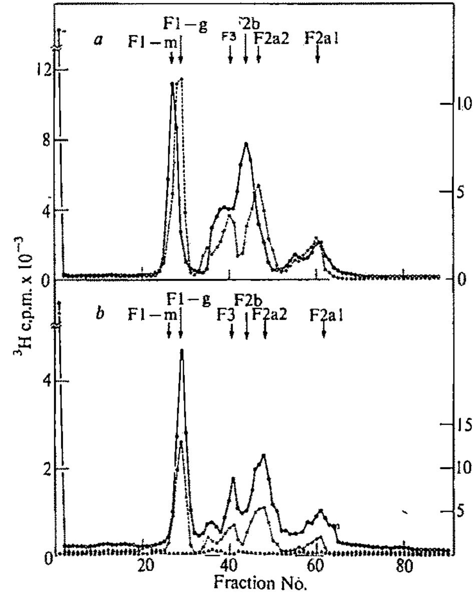

Fig. 3 Electrophoresis of products of cell-free protein synthesis on *p*H 2.7 acrylamide gels. 46 µl of the reaction mixture described in Table 1 was treated with DNase I (50 µg ml^{-1}) at 30° C for 20 min and then dialysed against 2.5 M urea, 0.9 N acetic acid, 0.2% 2-mercaptoethanol at 4° C. ^{14}C-lysine-labelled gastrula histones (*in vivo* marker) were added to the dialysed sample. The mixture was separated as described under Fig. 1. Only the histone-containing region is shown. *a*: ○—○, cell-free products directed by morula RNA; ● - - - ●, product of synthesis *in vivo* at gastrula stage. *b*: ○—○, cell-free products directed by gastrula RNA; ● - - - ●, gastrula marker histones; ▲ - - - ▲, cell-free products, no added RNA.

Total polyribosomal RNA was next prepared, purified, and translated in the ascites cell-free protein synthesis system employed by Gross *et al.*[16] for earlier studies on histone mRNA. Protein products of cell-free synthesis (Table 1), together with marker histones synthesised *in vivo* at the gastrula stage, were subjected to co-electrophoresis on acetic acid-urea gels. As is shown in Fig. 3*a*, RNA from morula polyribosomes directs the synthesis of F1-m. Total histones synthesised *in vitro* under direction of this RNA also give a high F2b/F2a2 incorporation ratio, which is characteristic of labelled histone that associates with chromatin *in vivo* at this stage of development. Gastrula polyribosomal RNA, by contrast, directs the cell-free synthesis of F1-g (Fig. 3*b*). The gastrula RNA-directed histones have the low F2b/F2a2 incorporation ratio characteristic of protein synthesis in the living embryo at this stage. In electrophoresis on SDS gels (Fig. 4), protein products made *in vitro* under the direction of morula RNA are again observed to contain only F1-m, while polyribosomal RNA from gastrulae yields products that separate on these gels as F1-g and a small amount of F1-m.

The postribosomal supernatant fraction of these embryos contains RNA with template activity in the cell-free system. Morula RNA of this kind directs the morula-specific pattern of lysine incorporation; little or no F1-g is labelled. Gastrula supernatant RNA contains sequences that encode and direct the synthesis of F1-g (data not shown here).

Polyribosomal (messenger) RNA from two different stages of development therefore contains sequences that direct histone synthesis *in vitro* according to patterns that match qualitatively, and to a considerable extent quantitatively, the corresponding patterns of synthesis *in vivo*. Mobility differences between the very lysine-rich histones encoded on and directed by the two kinds of template RNA are

Table 1 Cell-free Lysine Incorporation into Proteins Directed by Morula and Gastrula Polyribosomal RNA

Addition	^{3}H-lysine incorporated (c.p.m.)	Stimulation (X blank)
Blank (no RNA)	495	—
Morula RNA	6,126	12.4
Gastrula RNA	3,612	7.3

Polyribosomal RNA was prepared from 6 ml packed morulae and 8 ml gastrulae. Embryos were washed twice with $Ca^{2+}-Mg^{2+}$-free seawater, once with TNM (0.2 M NaCl, 5 mM Mg acetate, 10 mM Tris, *p*H 7.5), and homogenised in 2.5 volumes of TNM. The 12,000*g* supernatant was centrifuged through a 7–47% sucrose gradient for 3 h at 26,000 r.p.m. in a Spinco SW27 rotor. Polyribosomes were collected from appropriate fractions and their RNA extracted. This RNA was translated in the Krebs II ascites-derived cell-free system[16,22,23]. Input RNA was at 22.5 µg, prepared from morulae and gastrulae. The incubation system contained 10 µC ^{3}H-lysine at 20.8 Ci mmol^{-1}, plus 50 µM unlabelled amino acids (less lysine), in a volume of 50 µl. Incubation was for 60 min at 30° C. Two aliquots of 2 µl from each incubation were precipitated with hot 5% trichloroacetic acid and the precipitated radioactivity counted.

very likely to be due to differences in primary structure of the two proteins. The differences can in any case not be due to differential post-translational modification or derivatisation, since the information content of the two mRNA preparations is expressed through a constant enzymatic machinery, the components of the ascites cell-free incorporation system.

We conclude, therefore, that histones F1-m and F1-g of morulae and gastrulae, respectively, co-migrating as they do with corresponding products of cell-free synthesis directed by morula and gastrula RNA, also differ in their primary structures.

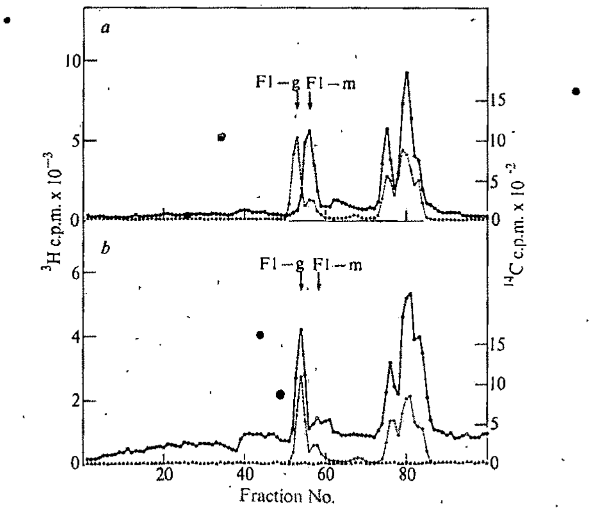

Fig. 4 Analysis of cell-free products on SDS-acrylamide gels. Morula or gastrula RNA was incubated with the cell-free protein synthetic system as described, and co-electrophoresis with ^{14}C-lysine gastrula marker histones was carried out as in the experiments of Fig. 2. *a*: ○—○, morula RNA-directed cell-free products; ●---●, gastrula marker histones. *b*: ○—○, gastrula RNA-directed cell-free products; ●---●, gastrula marker histones; ▲---▲, cell-free products, no added RNA.

Although other cases of histone tissue specificity are known[22,23], histone F1-g, which becomes detectable only after hatching in *Lytechinus* and other sea urchins[4,6], may be an example (so far rare) of a qualitatively new histone appearing in the course of early development. It is possible, of course, that traces of F1-g are present in pre-hatching stages and are not detected by methods such as were employed here, but this hardly affects the argument. Quantitative changes in the fractional synthesis of histones other than the F1 appear also to be regulated, at least in part, by the relative amounts of their corresponding messenger RNA in polyribosomes.

The situation in *Arbacia punctulata*, another species studied by the methods described above, is in essence the same as in *Lytechinus*, but with some minor modifications. Mobility relations between the two *Arbacia* F1 fractions differ from those of *Lytechinus* when the proteins are separated on acetic acid-urea gels. SDS gel electrophoresis reveals that the switch-over occurs earlier than in *Lytechinus*, and it seems to be completed by the late gastrula stage.

The data indicate, in summary, that the kinds and amounts of histones that associate with DNA in chromatin do in fact differ characteristically from one stage of development to the next and that the differences are in large part the result of differential messenger content of the translational machinery.

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Geographical Distribution of Australia Antigen Determinants d, y and w

AUSTRALIA antigen (Au) has a group of antigenic determinants on its surface, which may occur in different combinations in the sera of people living in different geographical locations. Several of these determinants have been characterised and given alphabetical designations[1–5]. It has been reported that determinants d and y tend to be mutually exclusive[6] although rarely they occur together (S. M. and S. B., unpublished). Determinants w and r also tend to occur in different samples rather than together and these two determinants have a strongly marked geographical distribution. Determinant w is common in the USA, South America, Europe and Africa, while r is common in Asia and the Pacific area[5,7]. We have tested 893 samples of Australia antigen from locations around the world for determinants d, y and w. Here we present these findings and speculate on factors which may contribute to the geographical distribution of Australia antigen subtypes.

The Australia antigen samples included in this study were all from apparently healthy asymptomatic carriers. They are part of the blood collection of the Division of Clinical Research at the Institute for Cancer Research and had previously been found to contain Australia antigen by immunodiffusion testing. Australia antigen determinants were identified using the standard seven hole immunodiffusion pattern cut in 1.1% agarose in pH 8.2 veronal buffer[8]. Specific typing sera were put in the centre well, control Australia antigen was put in the top and bottom wells, and test samples in the remaining four wells. Human anti-aw was reacted against an aw control antigen adjacent to the test samples. The formation of a line of identity between the control antigen and the test antigen was interpreted as indicating that the test antigen was w^+. When a spur was formed by the control antigen over the test antigen it was considered w^-. The same principle was used in testing for determinant d with guinea pig anti-ad antiserum (NIH V 801–502–058). A few sera which were known to be Australia antigen positive did not react with this serum at all. These will be considered in detail elsewhere, but here they were considered d^-. These and all other d^- sera were retested with a second anti-ad serum for confirmation of their d^- status. Absorbed chimpanzee anti-y and y control antigen were used to test for determinant y. Sera which were negative for both d and y were tested with an additional anti-ay serum.

The antigens have been grouped according to geographical origin of the sera and have been assigned to subtype categories suggested by Le Bouvier[6] according to the presence of determinants d and y (Table 1). Subtype A consists of those antigens which lack both the d and y determinants. This group is designated A because by definition it must have determinant a which is defined as the universal or group determinant. The subtype categories have been further divided into w^+ and w^-. In all but four cases, determinants d and y were found to be mutually exclusive. These are not shown in Table 1. Two of these four exceptions were found on Bougainville and the other two were from people living in the United States who had travelled in Asia.

Australia antigen with determinant d is common in asymptomatic carriers in the United States, North Europe, Asia and Oceania, although subtype y does occur in these areas. All antigen samples from Japan carry the d determinant while none carry y. Antigen with the y determinant occurs almost to the exclusion of d in Africa and in Australian aborigines. Determinant y occurs frequently in India and in the Mediterranean area in Italy and Yugoslavia.

Virtually all antigens from Europe, the United States, Africa, India and Australia carry the w determinant. Antigen without w predominates in the New Hebrides, Micronesia, New Caledonia, Bougainville, Japan, Malaita and Thailand (Table 2). Both w^+ and w^- occur in the population in China, the Philippines, French Polynesia, and New Guinea.

From these data and the data in Table 2, it seems that there are marked geographical separations of subtype. The basic northern European subtype is $adw^+ y^-$ and the African subtype is $ayw^+ d^-$. In the Mediterranean area these subtypes both occur concurrently. The Asiatic type seems to be mostly $adw^+ y^-$ with some $ad^+ wy^-$. Large areas of

Table 1 Distribution of w Determinant with Respect to Subtypes d, y and a in Asymptomatic Carriers from Various Geographical Locations

		D		Y		A	
	N	w^+	w^-	w^+	w^-	w^+	w^-
Northern Europe							
Belgium	2	2	0	0	0	0	0
Finland	31	31	0	0	0	0	0
Germany	4	2	0	2	0	0	0
Total	37	35	0	2	0	0	0
Mediterranean							
Turin, Italy	17	4	0	13	0	0	0
Yugoslavia	9	4	0	5	0	0	0
Total	26	8	0	18	0	0	0
Africa							
Ghana	6	0	0	6	0	0	0
Ivory Coast	3	0	0	3	0	0	0
Nigeria	5	0	0	5	0	0	0
South Africa (Africans)	2	1	0	1	0	0	0
Senegal	14	0	0	14	0	0	0
Total	30	1	0	29	0	0	0
Asia							
China	4	3	1	0	0	0	0
Japan	91	25	66	0	0	0	0
Philippines	5	5	0	0	0	0	0
Philippines (Cebu)	42	38	2	2	0	0	0
Thailand	3	2	1	0	0	0	0
Total	145	73	70	2	0	0	0
Far East							
India	20	2	0	16	1	0	1
Australia							
Aborigine	14	0	0	12	0	2	0
Oceania							
American Samoa	3	0	2	1	0	0	0
Bougainville							
North	34	0	34	0	0	0	0
South Central	172	17	75	42	35	1	2
Tairara	5	0	5	0	0	0	0
Total	211	17	114	42	35	1	2
French Polynesia							
Bora Bora	3	2	1	0	0	0	0
Tahiti	13	6	7	0	0	0	0
Tubai	1	0	0	0	0	1	0
Total	17	8	8	0	0	1	0
Malaita	63	2	61	0	0	0	0
New Caledonia							
Konmoi	2	0	1	1	0	0	0
La Foa	6	0	5	1	0	0	0
Noumea	5	3	1	1	0	0	0
Total	13	3	7	3	0	0	0
New Guinea							
Various groups	14	10	4	0	0	0	0
New Hebrides							
Aoba	8	0	8	0	0	0	0
Lamap	16	1	15	0	0	0	0
Norsup	1	1	0	0	0	0	0
Tanna	17	0	9	0	8	0	0
Vate	27	1	26	0	0	0	0
Total	69	3	58	0	8	0	0
Ponape	5	0	5	0	0	0	0
Rongelap	6	1	5	0	0	0	0
Utirik	7	0	7	0	0	0	0
Central and South America							
Brazil	3	1	0	2	0	0	0
Dominican Republic	19	11	0	8	0	0	0
Jamaica	2	0	0	2	0	0	0
Peru (Cashinahua)	35	33	1	0	0	1	0
Surinam	11	8	0	3	0	0	0
Yucatan	3	3	0	0	0	0	0
Total	73	56	1	15	0	1	0
North America							
Frobisher Bay, Canada	2	2	0	0	0	0	0
Philadelphia	22	18	0	4	0	0	0
Philadelphia (prisoners)	26	18	0	8	0	0	0
Red Cross donors	90	76	1	13	0	0	0
Total	140	114	1	25	0	0	0

Table 2 Distribution of Subtypes in Asymptomatic Persons Previously Described

Location	Subtype D	Subtype Y
Tonga[9]	30	11
Thailand[5]	21 *	2
Korea[5]	4 †	3
New Guinea[20]	9	0
Singapore (China)[20]	24	1
Sweden[21]	16	3
Germany ‡	30	2
Denmark[22]	89	5
Israel §	13	47
Iran[23]	0	23

* 2 ayw, 20 adr; 1 probably adw.

† 3 ayw 4 adr.

‡ A. Schober and R. Thomssen, personal communication.

§ S. Bar-Shany, V. M. Edwards, J. W. Mosley, and W. H. Bancroft, personal communication.

Oceania are ad^{+} wy^{-}. Japan is at a geographical interface with both adw^{+} y^{-} and ad^{+} wy^{-}. India and Australian aborigines are mostly ayw^{+} d^{-} and Melanesia is in the interface between Australia (ayw^{+} d^{-}) and the rest of Oceania (ad^{+} wy^{-}).

Figure 1 shows the distribution of dominant subtypes from different parts of the world tested in this study.

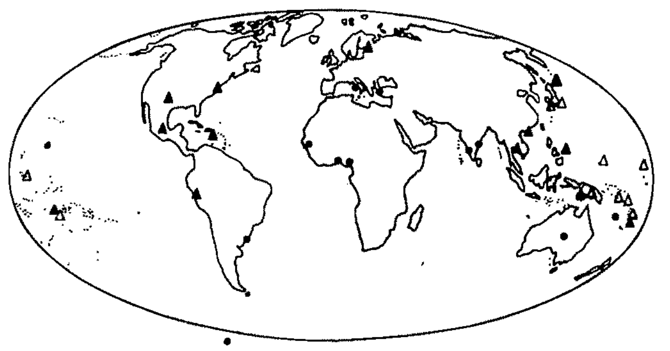

Fig. 1 Distribution of Australia antigen subtypes. Each symbol represents the dominant subtype in that location. Triangles represent subtype d. Circles represent subtype y. Solid figures represent w^{+}; open figures w^{-}.

One hypothesis generated by this study is that the Australia antigen subtypes among asymptomatic carriers could be used as population markers and serve as an indication of human migrations. This would be more likely if women were included in the migration since when women carry the antigen they can transmit it to some of their children who may then become chronic asymptomatic carriers[9-14]. When infants acquire Au from their mothers the subtype seems to be the same in both the mother and child[14,15]. Daughters can then transmit the same subtype to their children and so on through many generations. This could mantain the subtype brought by the migrant population in a new environment. These introduced subtypes might not predominate since there are other mechanisms which produce carriers, but they should persist to some degree.

The subtype ayw^{+} d^{-} is most common in Africa. In all areas tested where African slaves were introduced the ayw^{+} d^{-} subtype occurs but in lower frequency than in Africa. These areas include the USA, Brazil, Jamaica, the Dominican Republic and Surinam. This is in contrast to northern Europe, Japan and Oceania where there are few people of African descent and little ay antigen. In the United States where about 10% (ref. 16) of the population is considered to be of African descent, 15% of the carriers are ay[17]; while in the Dominican Republic where 85% of the people have some African admixture[18], 42% of the Australia antigen is ayw^{+} d^{-}. This hypothesis could be further tested by correlating subtype data with other biological data (blood and serum groups) and linguistic, archaeological and cultural information concerning human migrations. An Au subtype marker would be unique among known biological markers since it behaves as a unit and is passed from mother to child unaltered. In contrast, inheritable human biological markers are subject to Mendelian segregation and the resulting phenotypes can change rapidly from generation to generation.

Two United States chronic carriers were found to have w^{-} antigen. They had both been in Vietnam and were presumed to have acquired their exotic subtype in Asia where w^{-} is more common. We have tested a total of 318 Au samples from both patients and asymptomatic carriers in the United States and all except those two have had the w determinant. It is probable that some veterans of the Pacific theatre returned to the United States carrying ad^{+} wy^{-} at the end of the Second World War. This subtype has not spread in the United States population to any measurable extent. This is compatible with the assumption that maternal transmission is the major mechanism for preserving an introduced subtype in a new environment.

South Central Bougainville is unusual in that there are seven different Au subtypes. It is unlikely that this diversity could be due to chance. The diversity could be related to the fact that Bougainville is located between Australia where the subtype is entirely ayw^{+} d^{-} and Oceania where the complementary subtype ad^{+} wy^{-} occurs frequently. In areas such as Bougainville two subtypes could meet and recombine by a mechanism similar to crossover which may occur in a doubly infected host, either human, animal, or arthropod. For example, if ayw^{+} d^{-} and ad^{+} wy^{-} agents coexist in the same host it is possible that they could recombine and yield four types of Australia antigen; the original ayw^{+} d^{-} and ad^{+} wy^{-}, as well as ay^{+} wd^{-} and adw^{+} y^{-} recombinations. All of these do in fact exist in Bougainville and have not been found together in other locations.

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Silent Maternal Transmission of Australia Antigen

CHRONIC asymptomatic carriers of Australia antigen (Au, hepatitis B antigen, HBAg, HAA and so on) are found in human populations throughout the world and they constitute an important reservoir for the maintenance of the infectious agent. Here we provide evidence which, in combination with previous data, suggests that an important mechanism for the establishment of these carriers is through the 'silent' (that is, without illness) transmission of chronic infections from generation to generation through the maternal line.

There have been several studies related to mothers with Australia antigen and their children. We analysed 109 completed or partially completed families, from five groups [Cebu, Bougainville, Lau and Baegu speaking areas of Malaita, and Sardinian[1] families from Turin]. There was a much higher frequency of Au in the offspring of matings in which the mothers were asymptomatic carriers of Au and the fathers were not, than in matings in which the fathers were carriers and the mothers were not (for all the families combined $P=0.55\times10^{-10}$ (ref. 2). Okochi and his colleagues found that of twelve children born to Japanese mothers who were asymptomatic carriers of Au, seven children became carriers within the first 6 months of life[3]. Papaevangelou found that of eleven asymptomatic Greek mothers who had Au, two had babies who developed the antigen at 6 and 14 months, respectively. The antigen did not persist in these infants (G. Papaevangelou, personal communication). Turner *et al.* made a careful documentation of transmission of antigen from a mother with hepatitis to her infant[4]. Subsequently, the infants of five mothers with Australia antigen positive hepatitis were studied and four were found to be carriers of the antigen without clinical signs of hepatitis[5].

In a larger study of mothers living in California who had Australia antigenaemia during pregnancy, or within two months of the birth of their children, Schweitzer and his colleagues found a significant incidence of vertical transmission. Of twenty-three women who had hepatitis, twelve gave birth to infants who developed Australia antigenaemia, some of which began, not at delivery, but within weeks or months afterwards[6]. In each tested instance the babies had the same subtype antigen as their mothers[7]. The children did not have signs of hepatitis other than mild elevations of serum glutamic pyruvic transaminase (SGPT). Their development was normal although the antigenaemia has persisted for up to 23 months. Of fourteen mothers in this series who were chronic antigen carriers one gave birth to a baby who became a chronic carrier. The frequency of transmission from asymptomatic mothers varies in different locations. In contrast to Japan (7/12), Greece (2/11) and California (1/14), in Denmark[8] none of the babies of eighty-one carrier mothers became positive, and in Pakistan one out of eighteen infants of asymptomatic carrier mothers developed the antigen[9]. These studies indicate that Au transmission from mothers with hepatitis is common and in some places maternal transmission from chronic carriers does occur.

The source of Au infection sometimes can be identified by immunological studies. The surface of the antigen is a composite of determinants which are immunologically distinct[10–15]. Persons infected with material containing Au of a certain immunological combination, or subtypes, will develop those subtypes[16]. The same subtype persists in both hepatitis patients and chronic carriers for as long as they have the antigen, with rare exceptions[17,18]. We have used immunological subtyping to study relationships between Au carried by different members of the same family.

The sera used in this study were collected together with pedigrees in eighteen villages in south central Bougainville[19]. They represented 85% of the healthy residents over the age of 2 yr. It was assumed that asymptomatic people with antigenaemia were chronic carriers. There was an Au carrier rate of 10.2% on Bougainville and family clustering had been observed in the initial testing of these sera. The distribution of Au had previously been subjected to segregation analysis which was compatible with autosomal recessive inheritance[19]. The observed family clustering could also be explained by intrafamily transmission of an infectious agent or maternal transmission as suggested by Turner *et al.*[4]. If this were true, then all members of a family should have the same subtype. The presence of multiple subtypes in the same family would rule against the family infection hypothesis. Sera for testing were selected from families with more than one antigen carrier so that a comparison between family members could be made.

The subtyping was done by immunodiffusion in a standard seven-hole pattern cut in 1.1% agarose in veronal buffer, *p*H 8.2 (ref. 20). In preliminary testing for d and w the antiserum was put in the centre well and a control Au which carried the determinant complementary to the antiserum was put in the top and bottom wells. If a smooth line of identity was formed between the control antigen and the test antigen the test antigen was scored as positive for the determinant in question. If a spur was formed it was considered negative for the determinant. Absorbed antiserum was used to test for determinant y. Serum from a chimpanzee immunised with Au of subtype aywx was absorbed with adwx+y− antigen, leaving anti-y in the antiserum. This was used with a y positive antigen control. Antigens giving a line of identity were considered positive; those giving no line, negative.

A few unusual sera carried neither d nor y. All such d− y− antigens were retested with different dilutions of antisera and an additional anti-ad and anti-ay sera. All plates were dried and stained with azocarmine for better visualisation of precipitin lines.

Table 1 Australia Antigen Subtypes Found in Eighteen Villages on Bougainville

		Subtype D (d+y−)		Subtype Y (d−y+)		Subtype A (d−y−)		Subtype DY (d+y+)	
Village	Total Au.	w+	w−	w+	w−	w+	w−	w+	w−
1	18	0	3	10	4	1	0	0	0
2	4	1	1	2	0	0	0	0	0
3	3	0	2	0	1	0	0	0	0
4	5	0	1	3	1	0	0	0	0
5	5	0	4	0	1	0	0	0	0
6	14	0	9	5	0	0	0	0	0
7	14	4	8	0	2	0	0	0	0
8	22	6	10	0	5	0	1	0	0
9	4	3	0	1	0	0	0	0	0
10	19	1	15	2	0	0	1	0	0
11	2	0	1	1	0	0	0	0	0
12	11	0	1	10	0	0	0	0	0
13	2	0	0	1	1	0	0	0	0
14	5	0	2	0	3	0	0	0	0
15	5	0	0	1	3	0	0	1	0
16	1	0	0	0	0	0	0	1	0
17	20	1	11	0	8	0	0	0	0
18	20	1	7	6	6	0	0	0	0
	174	17	75	42	35	1	2	2	0

All possible combinations of determinants d, y and w except d+y+w− (Table 1) were found in this population. Of the 174 Au samples typed, two contained both d and y. This is a rare event and has not been reported before in normal people who have not received transfusions. These determinants seemed to be on the same particle (S. Mazzur and S. Burgert, personal communication).

Villages 2, 11, 13 and 16 did not contain families in which there were more than one member with Au. The data from families in the remaining villages which have more than one member with antigen are shown in Table 2. Of the thirty-two families tested there were eleven families in which two different combinations of antigens were segregating.

Asymptomatic carriers living in the same household can have different subtypes of Australia antigen (Table 2). This means that these carriers have not been infected by each other nor from the same source. This rules out simple household infection or maternal transmission as an explanation for all the observed family clustering in these families.

A special case of family infection does, however, seem to occur. In families where the mother was an asymptomatic carrier, the twenty-two children who were carriers with a single exception had the same subtype as their mother. Among the twelve families where the mother was positive, one had mixed subtypes, while among the twenty families in which the mother did not have Au, ten families had antigen of different subtypes. These reflected the subtypes in the general population of the village. Among the six families in which the father was positive, there were mixed subtypes in four of the families. Children with Au from families where either the mother but not the father, or the father but not the mother, carried the antigen were compared using Fisher's exact test (two-tailed). The difference in distribution was significant ($P=0.006$). When the families in which either parent was not available for testing were excluded from the analysis, the differences were still significant ($P=0.007$). The same test was used to compare the number of siblings of matching subtype in families with Au carrier and Au negative mothers. This difference was significant ($P=0.005$).

Table 2 Distribution of Australia Antigen Subtypes in Families where More than One Member has the Antigen in the Blood

Village	Father	Mother	Sibs				
1	0	w+d−y+	w+d−y+	w+d−y+	w+d−y+		
3	0	w−d+y−	0	w−d+y−	0		
6	0	w−d+y−	w−d+y−				
7	0	w+d+y−	0	w+d+y−	w+d+y−		
9	0	w−d+y−	0	w−d+y−	w−d+y−	0	
10	NA	w−d+y−	0	0	w−d+y−		
12	NA	w+d−y+	w+d−y+	w+d−y+	w+d−y+	w+d−y+	0
12	NA	w+d−y+	w+d−y+	0			
14*	0	w−d−y+	w−d+y−	0	w−d−y+		
17	NA	w−d+y−	0	w−d+y−	w−d+y−		
18	NA	w−d−y+	0	w−d−y+	0	0	
18	NA	w+d−y+	w+d−y+	0	w+d−y+		
8	w+d+y−	NA	0	w+d+y−			
8*	w−d+y−	0	w−d−y+	0			
8*	w−d+y−	0	0	w+d+y−			
8	w−d+y−	NA	w−d+y−	0			
10*	w−d−y−	0	0	w−d+y−	0	0	
17*	w−d+y−	0	w−d−†	0			
4*	0	0	0	0	0	w−d−y+	w+d−y+
5*	0	0	w−d+y−	w−d−y+			
6	NA	0	0	w−d+y−	w−d+y−		
6*	0	0	w+d−y+	w−d+y−			
7*	NA	0	w+d+y−	w−d+y−	w−d+y−	w−d+y−	w−d+y−
8*	NA	0	w−d+y−	w+d+y−	0	w−d+y−	0
10	0	0	w−d+y−	w−d+y−	w−d+y−	0	w−d+y−
10*	0	0	0	w+d+y−	w−d+y−	0	
12	NA	NA	0	w+d−y+	w+d−y+		
12	0	NA	w+d−y+	w+d−y+			
15	0	0	w−d−y+	w−d−y+			
17	0	0	w−d−y+	w−d−y+	w−d−y+		
18	0	0	w−d+y−	0	w−d+y−		
18	0	0	w−d+y−	0	w−d+y−	0	

* Indicates families with mixed subtypes. NA indicates not available.
† Insufficient amount to test for y.

These data indicate that vertical transmission from asymptomatic mothers to their asymptomatic children occurs in this environment. Horizontal transmission with the production of carriers must also occur within this population since maternal transmission cannot account for all of the observed carriers.

Genetic predisposition to the chronic asymptomatic carrier state has been proposed by Blumberg[19,21] and confirmed by Ceppellini[1] on the basis of segregation analysis of the distribution of Australia antigen in several populations. Segregation analysis of the distribution of Au in the same Bougainville sera which were used in this study revealed the segregation pattern expected of autosomal recessive inheritance. The vertical transmission reported in this communication is compatible with the genetic hypothesis since the genetic analysis was applied also to matings in which neither parent was a carrier of Au. There would be no maternal effect in these families. There is not an occult maternal effect due to undetected levels of antigen in the mothers' serum because the subtypes of the children do not, in general, match each other as they would in such a case.

Women who become Au carriers by virtue of genetic susceptibility can be expected to transmit the antigen to some of their children whether or not the children are homozygous for the postulated recessive Au susceptibility gene. Thus, a new maternal transmission line can be established from a genetically susceptible woman whose own mother was not a carrier provided the susceptible woman is exposed to infection. The interaction of the genetic predisposition to the carrier state and maternal transmission could be an important source of Au carriers. Further, the interaction of these two mechanisms, one of which is dependent on the survival and reproduction of women who are carriers, would provide a very rapid amplification of the number of carriers of Au in an environment where such a carrier state is advantageous. Such a selective advantage has been suggested by Blumberg *et al.*[22].

Not all children of mothers who are Au carriers become carriers themselves. There are factors, either genetic or environmental or both, which influence the outcome of the maternal-child relationship. We suggest that other, perhaps undiscovered, human pathogens survive by the mechanisms

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described here and are usually expressed as disease when passed horizontally or after long incubation. Techniques developed through the study of Australia antigen as well as epidemiological patterns disclosed by these studies could serve as models for the investigation of these agents. It has been suggested that other infectious agents may exist that have properties similar to Australia antigen. These have been termed Icrons[23].

Silent maternal transmission with the production of asymptomatic carriers seems to provide a reliable mechanism for virus survival particularly in small populations where the number of new susceptible individuals is limited. The carrier state maintains the infectious agent over long periods of time and the maternal-child interaction provides exposure of new susceptibles. In contrast, many acute viral infections spread quickly, exhaust the availability of susceptible hosts and disappear from isolated populations.

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Lethal Effect of Near-ultraviolet Irradiation on Mammalian Cells in Culture

THE lethal and mutagenic effects of ultraviolet light of wavelength below 300 nm on bacterial and mammalian cells have been the subject of many research projects, and several laboratories are investigating the action of near-ultraviolet irradiation provided by black light (300 to 420 nm) on bacterial cells[1]. There are, however, only a few studies on the effect of black light (near-ultraviolet) on mammalian cells[2,3]. Several laboratories have reported effects of both visible fluorescent light, which does produce a significant amount of emission in the near-ultraviolet region, and black light on mammalian cells after the incorporation of bromodeoxyuridine (BUdR) into the cell nucleic acid[3–7].

Experiments were initiated in this laboratory to study the effect of black light on mammalian cells in culture. Here we report that when mammalian cells in tissue culture medium were irradiated with black light, the cells were killed both reproductively (as evidenced by measuring clone-forming ability) and physiologically (as evidenced by staining with trypan blue dye).

The cells used were mouse 3T6 (ref. 8), Chinese hamster V79 clone 753-B-3M (ref. 9) and human D98/AH$_2$ (ref. 10). The cells were cultured in Dulbecco's modification of Eagle's medium[11] supplemented with 10% calf serum but without addition of antibiotics. The CO$_2$ tension for cell growth and light exposure was held at 10%. The light sources used were two General Electric F15T8 BLB integral filter black light tubes with emission in the 290 to 420 nm range peaking at 365 nm[12]. Light exposures were carried out in covered Falcon 3002 60 mm dishes containing 3.5 ml medium with no phenol red or serum. The culture dish lids excluded irradiation below 300 nm[13]. Exposure intensity was 5 μW mm^{-2} on the culture dish lid and 4 μW mm^{-2} at the cell surface as measured by a Blak-ray meter J-221 (Ultraviolet Products, Inc.). Temperature was monitored and regulated to avoid any effect due to heating by the light source.

When mouse 3T6 fibroblast cells plated at 120 and 10^4 cells per 60 mm dish were exposed to the BLB lights, 90% and 99% of the cells lost the ability to form clones during exposure times of 90 and 130 min respectively (Fig. 1*a*). The plating efficiency decreased less rapidly if the initial inoculum was 10^5 cells. When 10^6 cells were plated, no appreciable loss of plating efficiency was detected.

Similar density-dependent loss of plating efficiency was found with Chinese hamster V79 and human D98/AH$_2$ cells (Fig. 1*b* and *c*). V79 cells were inactivated at a slower rate than 3T6 cells when 120 and 10^4 cells were plated per dish. No loss of plating efficiency was detected in dishes plated with 10^5 or 10^6 cells. D98/AH$_2$ cells were as sensitive to BLB as 3T6 except that the loss of plating efficiency was more rapid when 120 cells were plated per dish.

It seems that the final slopes of the inactivation curves, where loss of plating efficiency did occur, were similar for each cell line. The shoulders in the curves became more pronounced as cell density was increased.

The above experiments measured the loss of the cells' ability to form clones, that is, the amount of reproductive death. In order to ascertain whether black light killed the cells physiologically as well, we measured the ability of the cells to exclude the dye trypan blue which stains physiologically non-viable cells. Dishes containing 3,000 cells each were exposed to black light at 4 μW mm^{-2} for 90 min and returned to a dark incubator. At various intervals after the exposure, trypan blue was added and the number of cells without dye was counted.

When 3T6 cells were used, approximately 90% of the cells lost their ability to exclude the dye during a 2 to 4 h period (Fig. 2*a*). For V79 cells the loss occurred between 6 to 8 h

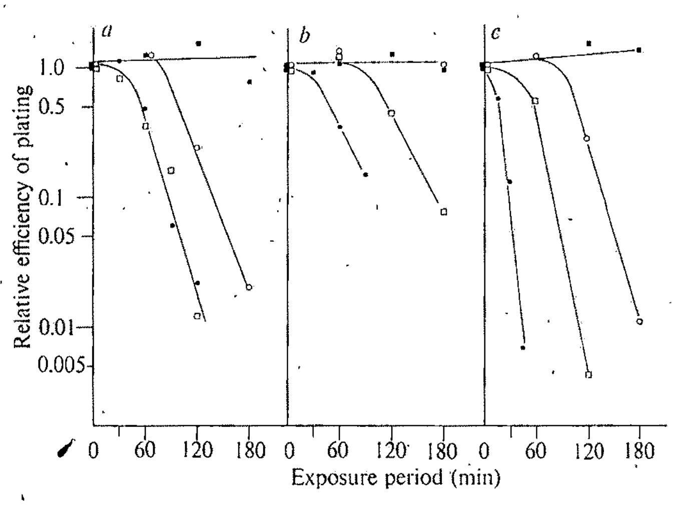

Fig. 1 The cells were trypsinised and plated in medium containing serum, but no phenol red, 18 h before exposure to 4 μW mm^{-2} black light. Twenty minutes prior to irradiation, the medium was removed and replaced with medium free of both phenol red and serum. The dishes were placed in the exposure chamber under 10% CO_2 for 20 min before the light was turned on. At various intervals dishes containing 120 cells were removed from the light and medium was replaced with fresh medium with serum. Dishes with 10^4, 10^5 and 10^6 cells were subcultured and diluted into dishes containing fresh medium with serum. The subculture procedure itself does not alter the viability of the cells. All dishes were incubated at 37° C for 7 to 10 d in the dark. The dishes were then stained with haematoxylin and colonies counted. The result is expressed in relative efficiency of plating, or the number of colonies formed divided by number of cells inoculated initially, with the zero time value set equal to 1. ■, 10^6; ○, 10^5; □, 10^4; ●, 120 cells plated per 60 mm dish and exposed to black light. *a*, 3T6 cells; *b*, V79 cells; *c*, D98/AH_2 cells.

(Fig. 2*b*). We expected that at least 99% of the 3,000 D98/AH_2 cells would be killed after the 90-min exposure period (Fig. 1*c*) and the same proportion of cells (>99%) actually did lose the ability to exclude trypan blue within a 10-h period after exposure to black light (Fig. 2*c*).

Our results showed that black light killed human, mouse and Chinese hamster cells in Dulbecco's medium. Human and mouse cells were killed when up to 10^5 cells were plated per 60 mm tissue culture dish, and Chinese hamster cells were killed at densities up to 10^4 cells per dish. The killing effect was density dependent. When 10^6 cells were plated, no decrease in viability was observed.

At the molecular level, the effects of far-ultraviolet (< 300 nm) and near-ultraviolet (300 to 420 nm) on biological systems are quite different[14]. Far-ultraviolet leads to lethality by directly altering the DNA through dimer formation. Near-ultraviolet, at the dosage used in these experiments, does not produce dimers in bacterial systems[1]. A possible chromophore may be present in the respiratory chain which absorbs energy in wavelengths above 300 nm[14]. Recombinationless bacterial mutants are highly sensitive to near-ultraviolet, suggesting that DNA may be an indirect target of the action of near-ultraviolet[1]. One cannot rule out other possible chromophores which may act indirectly to produce the results reported here. It is also conceivable that toxic photoproducts are produced in the medium which when taken up by the cells leads to lethality. Our finding that the cell killing effect is density dependent could be due to the fact that when more cells were present, the amount of photoproducts absorbed by each cell was correspondingly less. This possibility is compatible with the shoulders observed in the inactivation curves (Fig. 1). No cell killing would be observed until the increase in toxic products was adequate to saturate each cell to a level sufficient to inactivate it. In a separate manuscript (unpublished) we shall show that after components of tissue culture medium are exposed to near-ultraviolet, toxic photoproducts are formed which are primarily responsible for the type of mammalian cell inactivation as reported here.

The black light tubes used in this study emit in the 290 to 420 nm region. Although the peak of emission is relatively sharp[12], the responsible wavelengths for the killing observed here remain uncertain. Experiments are currently in progress with monochromatic light to determine the wavelengths which are most effective.

A better understanding of the effect of light irradiation in the 300 to 420 nm region on mammalian cells is needed. Visible fluorescent light, which illuminates virtually every laboratory, contains a significant amount of emission in this region. Puck and Kao[4] devised a technique utilising BUdR and visible fluorescent light for the selection of auxotrophic Chinese hamster mutant cells after treatment with mutagens. Chu *et al.*[3] reported that such selective techniques had highly mutagenic action. Visible fluorescent light increased the amount of polyoma virus induction nine-fold[7]. The near-ultraviolet region of visible fluorescent light can kill mammalian cells in culture (unpublished). The finding that near-ultraviolet produced by black light can effectively kill mammalian cells in tissue culture medium, as reported here, further emphasises the fact that considerable caution should be exercised in experiments involving the irradiation of mammalian cells with visible fluorescent light or black light. In addition, since black light is widely used outside the laboratory, the possible mutagenic and carcinogenic action of 300 to 420 nm radiation also needs to be carefully examined.

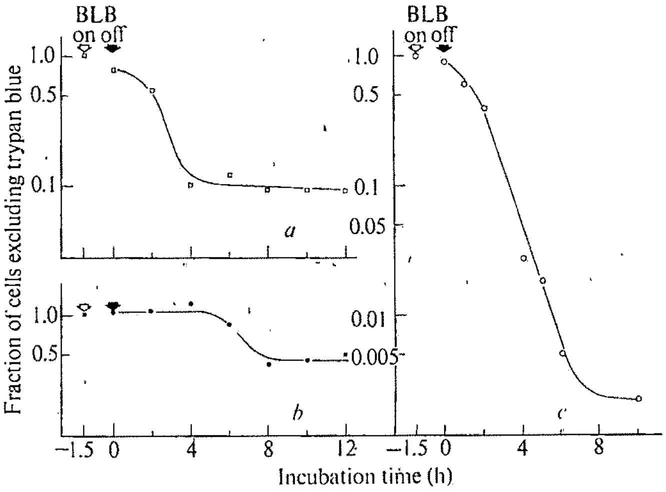

Fig. 2 3,000 cells were plated per 60 mm dish and exposed to 4 μW mm^{-2} black light as in Fig. 1. The cells were exposed to light for 90 min before being returned to incubation in the dark. At various intervals, trypan blue (0.5%) was added to the dishes for 5–7 min and the number of cells containing no dye was counted under ×40 magnification. Symbols as in Fig. 1; *a*, 3T6 cells; *b*, V79 cells; *c*, D98/AH_2 cells.

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Control of Cell Surface Topography

According to the fluid mosaic model of membrane structure[1], surface proteins are free to diffuse in a lipid matrix and thus to assume a random or homogenous distribution over the cell surface. In support of this model it has been shown that the distribution of glycoprotein receptors for the plant lectin concanavalin A (con A) is random in all cell types studied. In a variety of living systems it has also become clear that surface elements can be induced by exogenous agents to assume a non-random or heterogeneous distribution; for example, in virus transformed cells con A induces a clustering of con A binding sites. Recently, we and others have shown that cellular components sensitive to colchicine alkaloids (microtubules and perhaps other structures with similar pharmacological specificities) can affect the topography of certain surface elements. We shall review here the experimental evidence for systems in which topographical heterogeneity of specific surface elements (lectin binding sites and membrane transport carriers) can be induced and can be altered by colchicine. We shall then advance a general hypothesis involving specific interactions between surface proteins and intracellular colchicine binding proteins (CBP), which rationalises some apparent contradictions in this evidence and which indicates a molecular process at the level of the plasma membrane by which cells may respond to extracellular substances.

(1) When virus transformed fibroblasts are fixed with paraformaldehyde, the distribution of con A binding sites is random, as it is in non-transformed cells and as predicted by the fluid mosaic model. When con A is adsorbed to cells before fixation the distribution of con A binding sites in non-transformed cells is, however, still random, whereas in transformed cells the binding sites are gathered into clusters[2]. These clusters are withdrawn from the edges of the cell but otherwise are themselves disperse (T. E. U., R. D. B., J. Z. Borysenko, and M. J. Karnovsky, unpublished). Incubation of transformed cells with 1 μM colchicine before con A does not affect the formation of clusters, but the clusters are now loosely grouped into a large circumnuclear surface aggregate. These data suggest an enhanced mobility of con A binding sites after transformation which is further increased by alkaloid treatment.

(2) Treatment of lymphocytes with low concentrations of con A at 37° C leads to movement of con A binding sites into large aggregates known as "caps". At high concentrations of con A, however, no caps are formed during incubation at 37° C[3,4]. Thus, con A binding sites seem to be immobilised after addition of the high concentrations of lectin. In parallel experiments where lymphocytes are cooled to 4° C (presumably dissolving microtubules) or treated with colchicine, con A leads to capping even at high concentrations[5], indicating an increased mobility of con A binding site clusters in these cells.

(3) During phagocytosis particles are enveloped by cytoplasmic membrane which is then withdrawn into membrane-bound intracellular vesicles. Under suitable conditions more than 50% of the surface of polymorphonuclear leukocytes or alveolar macrophages can be internalised in this fashion. Using initial rates of purine and amino acid transport to assay the numbers of specific carrier proteins in the membrane, it has been shown that the same number of transport carriers are present before and after removal of a high proportion of the plasma membrane by phagocytic ingestion of latex beads[6]. Other experiments[6] rule out the insertion of new carriers into the surface membrane. This preservation of transport sites indicates their separation into a topographically distinct area which does not undergo internalisation. When leukocytes are pretreated with colchicine, however, phagocytosis leads to a decrease in transport rate which parallels the degree of membrane internalisation[7]. Thus, it seems that the forces which exclude transport carriers from phagocytic areas do not operate after colchicine treatment.

(4) Morphological studies of non-phagocytosing leukocytes show that con A binding sites are distributed randomly on the cell surface (J. M. O., R. D. B., J. Z. Borysenko, and M. J. Karnovsky, unpublished). When con A and *Ricinus communis* agglutinin (RCA) receptors are titrated after the phagocytic ingestion of droplets of a paraffin oil emulsion it can, however, be shown that the specific activity of surface binding sites (defined as μg lectin bound per 100 μg membrane protein) is reduced by 40–60% (the reverse of case (3) above for transport proteins[8]). Thus, during phagocytosis lectin binding sites are removed from the surface, apparently by concentration in the regions undergoing internalisation. After colchicine treatment, the removal of specific populations of lectin binding from the surface is greatly reduced, indicating an uncoupling of the process which links the concentrative movement of con A binding sites with the internalisation of phagocytic particles[9].

The effects of colchicine on (1) and (2) as compared with (3) and (4) on movement of specific surface elements are in apparent contradiction. Thus, movement of con A binding site clusters induced by con A, as in cap formation, occurs only after colchicine treatment whereas the concentrative movements of transport carriers and lectin binding sites induced by phagocytosis do not occur after colchicine. This paradox can be resolved simply by the following hypothesis: for cross-linking by con A (1) and (2), clusters of con A binding sites must have sufficient mobility to be approximated by the lectin. This requires release from cellular restraints which tend to separate and/or direct them. Colchicine abolishes these restraints. On the other hand, the directed movements which occur during phagocytosis ((3) and (4)) require the attachment of receptors (or transport proteins) to these restraining cellular structures which themselves direct specific movement. Colchicine abolishes these attachments and thus the cell-directed movement of con A binding sites into (and probably certain transport proteins out of) membrane internalised by phagocytosis. Thus, in all cases colchicine abolishes the attachment of membrane proteins to mobile cellular structures, permitting their association by exogenous cross-linking agents on the one hand, but abolishing cell-directed movements on the other.

What is the nature of these colchicine-sensitive linking structures? In the cases so far examined their pharmacological specificity parallels that of microtubular proteins: sensitivity to colchicine, colcemid, and the chemically unrelated vinblastine, and insensitivity to the photochemical derivative, lumicolchicine. Operationally, therefore, the 'linkage element' behaves like a microtubular protein, but it is not yet proven that colchicine treatment alters membrane properties through its interaction with structures identified morphologically as microtubules. It is possible that contractile structures, perhaps microfilaments, may interact with these colchicine-sensitive elements in order to facilitate movements of the surface proteins.

Our hypothesis represents a synthesis of the fluid mosaic theory, which suggests a random distribution of surface proteins, with data from several experimental systems in which topographical heterogeneity can be produced. It is proposed that con A-receptor complexes can be aggregated into large caps if the receptors are not restrained in their movement patterns by colchicine-sensitive elements; and in addition that movement of con A binding sites can be directed into local regions by exogenous agents such as phagocytic particles if these linkage elements are intact, that is, cell-directed movements of con A binding sites and cross-linking by bound con A are in this sense mutually exclusive. Experiments which test this theory may allow more precise definition of the mechanisms by which changes in cell surface topography can be directed by intrinsic cellular structures in response to extrinsic forces.

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Immune Complexes induce Selective Release of Lysosomal Hydrolases from Macrophages

IMMUNE complexes contribute to tissue damage in a variety of experimental and human diseases. Immune complexes induce arthritis[1] and nephritis[2,3] in laboratory animals while lupus glomerulonephritis[4] and possibly rheumatoid arthritis[5] are examples of their effects in man. The mechanisms by which damage is initiated and perpetuated are poorly understood. In the acute stage of inflammation resulting from immune complexes many granulocytes are present. These cells have abundant lysosomes rich in acid hydrolases which when released can degrade various tissue components. Enzyme release may result from cell death or exocytosis of granules from viable cells. Henson[6] and others[7,8] have shown that granulocytes exposed to immune complexes *in vitro* release lysosomal enzymes while remaining viable, and presumably the same happens in Arthus reactions *in vivo*.

After the initial phase of immune complex-induced tissue injury, neutrophils are no longer prominent and other cells may play a role in the continuation of the inflammation.

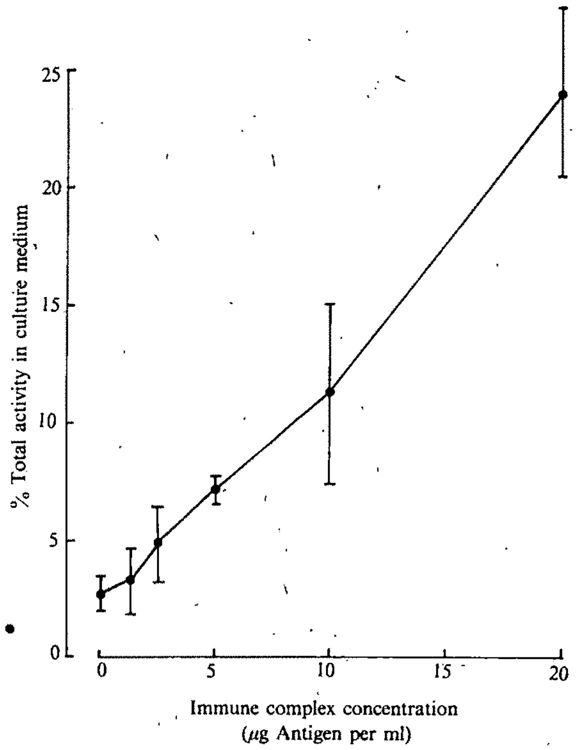

Fig. 1 The release of β-glucuronidase from macrophages exposed to increasing concentrations of immune complexes. Macrophages were collected by peritoneal lavage of outbred Swiss mice (LACA strain, SACI, London Road, Braintree, Essex) with 5 ml M199 (Grand Island Biological Co., Grand Island, New York)+10% swine serum (Biocult Laboratories, Glasgow). 2 to 2.5×10^6 cells were plated in 48 mm plastic Petri dishes (Nunc, Jobling Laboratories Division, Stone, Staffordshire). After 30 min the cell sheets were washed four times with phosphate-buffered saline and then cultured in M199 +10% swine serum at 37° C in a 5% CO_2 in air atmosphere for 48 h. Immune complexes were formed by interacting ferritin (Miles Laboratories) and rabbit anti-ferritin serum (Miles Laboratories). The point of equivalence was determined by quantitative precipitation[16]. The cells were exposed to increasing amounts of immune complexes for 6 h. At this time the culture medium was removed, centrifuged at 1,000 r.p.m. for 10 min in a No. 59548 swing-out head of a Mistral 6L centrifuge to pellet detached cells. The cell sheet was washed once with 0.9% NaCl (w/v) and then removed in 2.5 ml 0.9% NaCl (w/v) containing 0.1% Triton X-100 (w/v). All fractions were assayed for β-glucuronidase activity by the method of Talalay *et al.*[17]. Each value represents the mean ± s.d. of four separate plates.

Phagocytic cells such as blood-borne monocytes, tissue macrophages, mesangial cells of the kidney and A cells of synovium are present at sites of tissue damage in chronic inflammation associated with immune complexes. Moreover, mononuclear phagocytic cells are known to interact with immune complexes. Human monocytes have receptors for the Fc portions of IgG1 and IgG3[9]. Mouse peritoneal macrophages and rabbit alveolar macrophages take up immune complexes[10,11], as do renal mesangial cells[12] and A cells of rheumatoid synovium[1]. The interaction of these phagocytic cells with immune complexes may also result in tissue damage. These cells contain lysosomal enzymes which can not only digest immune complexes but degrade connective tissue constituents when released from the cells[13]. Lysosomal hydrolases may again be released from macrophages by cell death[14] or selectively discharged from viable cells (ref. 15 and P. D., R. Page, and A. C. A., unpublished). It has already been reported that macrophages take up and digest immune complexes *in vitro*[10]. We now present evidence that this process is accompanied by a selective release of macrophage hydrolases from viable cells into the culture medium.

Figure 1 shows the effect of increasing concentrations of an immune complex on the release of lysosomal enzymes from macrophages. After 6 h incubation in medium containing 5 $\mu g\ ml^{-1}$ of immune complex, the concentration of β-glucuronidase in the medium is significantly greater than controls ($P<0.01$). There is a dose-dependent release of enzyme rising to 24.0±3.5% with 20 $\mu g\ ml^{-1}$ of immune complex.

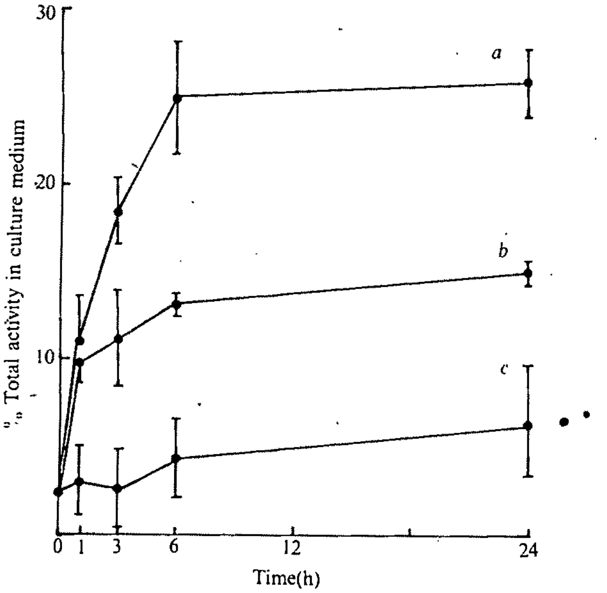

Fig. 2 The time dependence of macrophage β-glucuronidase release induced by immune complexes. The experiment was carried out in similar conditions to those described in Fig. 1 except that immune complexes were formed at equivalence from bovine serum albumin, Cohn Fraction V (Sigma Chemical Co., London) and rabbit anti-bovine serum albumin (BSA–anti-BSA). The latter was prepared by intramuscular inoculation of New Zealand White rabbits with 15 mg of antigen followed 3 weeks later by injection of 5 mg antigen intravenously. The animals were bled 10 d later. Each value represents the mean ±s.d. of four separate plates. *a*, 20 $\mu g\ ml^{-1}$; *b*, 10 $\mu g\ ml^{-1}$; *c*, control.

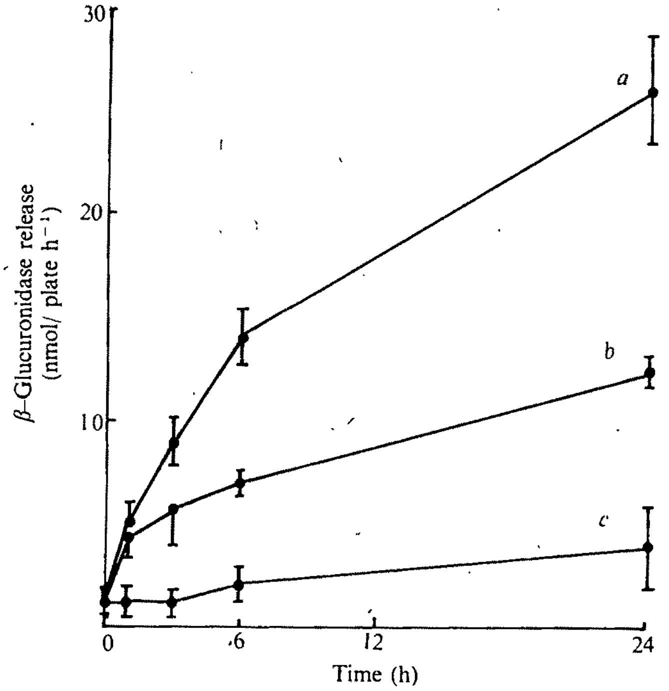

Fig. 3 The release of increasing amounts of β-glucuronidase from macrophages exposed to immune complexes for 24 h. Experimental conditions were identical to those described in Fig. 2. Each point represents the mean ±s.d. of four separate plates. *a*, 20 $\mu g\ ml^{-1}$; *b*, 10 $\mu g\ ml^{-1}$; *c*, control.

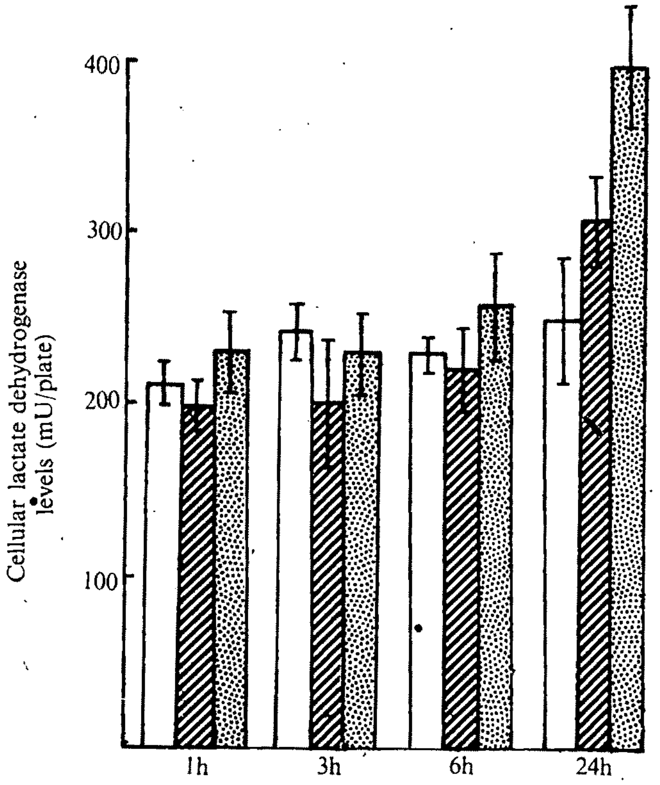

Fig. 4 The levels of lactate dehydrogenase (LDH) in macrophages exposed to immune complexes. Experimental conditions were identical to those described in Fig. 2. LDH was assayed using Biochemical Test Combination LDH-UV (Boehringer Corp. (London) Ltd). □, Control; ▨, immune complexes (20 $\mu g\ ml^{-1}$); ▒, immune complexes (10 $\mu g\ ml^{-1}$).

Release of the lysosomal enzyme is time dependent. In Fig. 2, the release of β-glucuronidase into the culture medium over 24 h with 10 $\mu g\ ml^{-1}$ and 20 $\mu g\ ml^{-1}$ of immune complex is shown. Release is demonstrable 1 h after contact and continues over the subsequent 23 h. Although the percentage of total β-glucuronidase activity in the culture medium plateaus after 6 h, the absolute amount continues to increase because of enzyme synthesis by the cells (Fig. 3). This indicates that depletion of intracellular acid hydrolase may act as a signal for increased enzyme synthesis.

Figure 4 shows that at no time during the 24 h period is there a significant decrease in cellular lactate dehydrogenase levels, compared with controls, of cultures exposed to immune complex ($P>0.01$) in all instances. Moreover, cultures exposed to 10 $\mu g\ ml^{-1}$ of immune complex for 24 h show a statistically significant ($P<0.01$) increase in cellular LDH level compared with its control.

For the sake of brevity we have only presented data for β-glucuronidase, but a similar pattern of selective release has been demonstrated for other lysosomal hydrolases such as N-acetyl-β-D-glucosaminidase and β-galactosidase; this information will be published in detail elsewhere.

It is now clear that both granulocytes and macrophages, phagocytic cells associated with acute and chronic inflammation respectively, can selectively release lysosomal hydrolases with no detectable loss of viability. This phenomenon may provide an explanation for the ability of macrophages to cause tissue damage and degradation at sites of chronic inflammation while retaining their viability over prolonged periods of time. This interpretation is supported by evidence that other agents inducing chronic inflammation, such as Group A streptococcal cell walls, also cause selective release of lysosomal hydrolases from viable macrophages *in vitro* (P. D., R. Page, and A. C. A., unpublished).

As immune complexes and macrophages are both present in the joints of patients with rheumatoid arthritis, release of macrophage hydrolases could play an important part in degradation of cartilage and other connective tissue changes observed in this disease. The role of immune complexes and macrophages in other chronic inflammatory conditions also deserves further study.

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Vitamin A Deficiency enhances Binding of Benzo(*a*)pyrene to Tracheal Epithelial DNA

THE relationship between carcinogen action and the state of cellular differentiation in target tissues is still unclear. Vitamin A controls the state of differentiation in many epithelial tissues[1], including those of the respiratory tract[2,3]. Vitamin A can also modify the process of carcinogenesis in many tissues; in a variety of experiments, the results obtained have often been influenced by experimental variables such as the animal species, the time and route of vitamin A administration, and the morphological type and the tissue site of tumour induced[4-10]. In the respiratory epithelium[3] vitamin A deficiency causes basal cell hyperplasia and squamous metaplasia. In order to study the possible relationship of the altered state of differentiation of respiratory epithelium to chemical carcinogenesis in this tissue, we have studied the binding of tritiated benzo(*a*)pyrene (^{3}H-BP) to DNA in tracheal epithelial cells of normal and vitamin A deficient hamsters. The binding of carcinogens to DNA has been indicated as a potentially crucial step in the carcinogenic process[11-13]. For polynuclear hydrocarbons such as benzo(*a*)pyrene, this binding is presumed to be the ultimate result of metabolism to reactive carcinogenic forms[14-16]; the extent of binding may be a measure of the carcinogenic potential towards a given tissue.

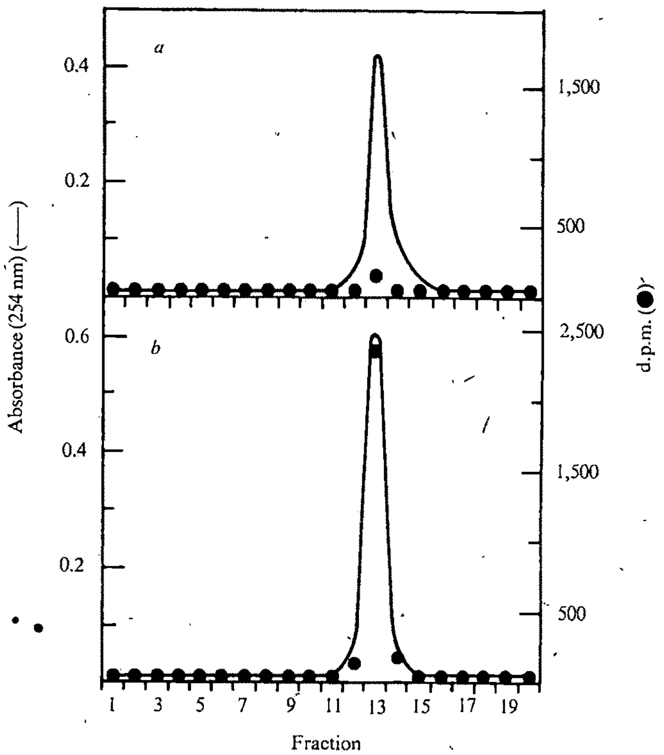

Fig. 1 CsCl gradient profiles of DNA purified from tracheal epithelium isolated from, *a*, normal and, *b*, vitamin A deficient hamsters. Isolated tracheas were maintained *in vitro* in L-15 medium containing 8.0×10^{-7} M ^{3}H-BP (20 μCi ml^{-1}, specific activity 25 Ci mmol^{-1}, Amersham-Searle). Following 3 h incubation, the tracheal epithelial cells were scraped from supporting structures and a crude preparation of DNA was obtained by phenol extraction and precipitation with 50% ethanol. The precipitate was extracted with ether, benzene, and chloroform-methanol (3 : 1), and then sequentially digested with RNase (Worthington Biochemicals) and Pronase (Calbiochem). The aqueous solution of DNA was extensively dialysed and then banded on CsCl gradients (Beckman SW56 rotor, 35,000 r.p.m., 66 h, 25° C). Gradients were fractionated into 0.2 ml portions, and absorbance and radioactivity were determined on each fraction. The absorbance of the gradient effluent was continuously monitored at 254 nm, as shown by the continuous line; radioactivity (d.p.m.) is indicated as points.

We report here the relative binding of ^{3}H-BP to tracheal epithelial DNA of vitamin A deficient and pair-fed normal hamsters under conditions in which isolated tracheas were incubated *in vitro* in the presence of ^{3}H-BP. Hamsters were made vitamin A deficient by maintenance on a vitamin A deficient diet starting at day 10–14 after birth; controls were pair-fed with the same diet supplemented with vitamin A (100 μg *all-trans* retinyl acetate, twice a week, by stomach tube)[3]. Tracheas were removed from the vitamin A deficient and pair-fed control hamsters and the isolated tracheas were incubated *in vitro* in L-15 medium containing ^{3}H-BP under conditions which preserved biochemical function and morphology[17]. Following incubation, the tracheas were washed extensively with phosphate buffered saline and the tracheal epithelial cells were scraped from supporting structures. DNA was isolated from the epithelial cells, and the purified DNA was banded in CsCl gradients as previously reported[18]. The results are shown in Fig. 1 and Table 1. Substantially greater quantities of radioactivity were associated with DNA prepared from vitamin A deficient tracheas. When isolated tracheas were incubated *in vitro* in the presence of ^{3}H-BP and equimolar amounts of 7,8-benzflavone (7,8-BF), an inhibitor of the aryl hydrocarbon hydroxylase system[19,20], the binding of ^{3}H-BP to DNA was reduced (Table 1). This result strongly suggests that the extent of benzo(*a*)pyrene binding to DNA depends largely on the ability of tracheal epithelial cells to metabolise it. The extent to which the increased binding of benzo(*a*)-pyrene to tracheal DNA in vitamin A deficient animals depends on altered activity of microsomal aryl hydrocarbon hydroxylase enzymes or on other altered cellular conditions, such as altered cellular membrane permeability or increased DNA synthesis, has yet to be established.

Table 1 Specific Activity of ^{3}H-BP bound to DNA of Tracheal Epithelial Cells during *in vitro* Incubation

Vitamin A status of animals	Addition to incubation medium ^{3}H-BP Exp. 1	^{3}H-BP Exp. 2	^{3}H-BP and 7,8-BF Exp. 2
Normal	6.0	13.4	1.3
Deficient	25.0	76.2	1.3

The data are expressed as d.p.m. per µg DNA. The µg of DNA and d.p.m. was determined from the peak DNA fraction of CsCl gradients. For each value presented in the table, DNA was prepared from two tracheas and pooled before purification on CsCl gradients. The animals were made vitamin A deficient by maintenance on a vitamin A deficient diet, starting on day 14 after birth in Exp. 1 and on day 10 after birth in Exp. 2.

The cellular location of the increased binding of benzo(*a*)-pyrene in vitamin A deficient tracheal epithelium has recently been demonstrated by quantitative light microscope autoradiographic studies. C. C. H. *et al.* (unpublished observations), using quantitative techniques[21], have demonstrated that in vitamin A deficient tracheas there is a higher binding of ^{3}H-BP to macromolecular structures of basal cells located in squamous metaplastic areas than to basal cells located in normal areas. These studies will be reported elsewhere in full detail.

The aim of the above experiments was to study the effects of a potentially co-carcinogenic condition, namely vitamin A deficiency, on the extent of binding of benzo(*a*)-pyrene to hamster tracheal DNA. It has not yet been shown in parallel long term animal studies, however, that vitamin A deficiency determines a state of high carcinogenic susceptibility in this tissue and in this species. In an analogous *in vivo* experiment conducted on rats which had been treated with the carcinogenic hydrocarbon 3-methylcholanthrene, preliminary results suggest that there is a higher incidence or an earlier appearance of respiratory tract tumours in animals fed a diet with a subnormal vitamin A content, compared with animals maintained on a normal vitamin A diet (P. Nettesheim, personal communication). Similar results have also been reported for colonic tumour induction in rats maintained on diets with subnormal vitamin A content[22,23]. The above results suggest that determination of the extent of binding of benzo(*a*)pyrene to DNA during short term organ culture of isolated tracheas may be applicable as a method for screening potential states (or potential individuals) with increased susceptibility to respiratory carcinogenesis.

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Evidence for a Junctional Effect of Lead on Neuromuscular Function

The peripheral neuromuscular pathology of lead has been extensively described in the clinical literature, but the mechanism of lead toxicity which results in neuromuscular impairment is not known[1]. Early work attempted to locate the site of a toxic lesion in the muscle and showed changes in muscle inorganic phosphate and creatine phosphate levels after lead treatment[2,3]. Experiments on the superior cervical ganglion, however, suggested that lead affected synaptic transmission by lowering the amount of acetylcholine released on preganglionic stimulation[4]. In order to separate the effects of lead on nerve from effects on muscle we have carried out experiments using the isolated phrenic nerve-diaphragm preparation.

Male Sprague-Dawley rats, weighing 120 to 135 g, were anaesthetised and the diaphragms with both phrenic nerves attached were removed and separated into two hemidiaphragms, each with a nerve trunk attached. The nerve-muscle preparation was similar to that originally described by Bulbring[5], modified so that preparations could be stimulated separately through either the nerve or muscle. One hemidiaphragm with its phrenic nerve was exposed to lead, as lead chloride, in a range of concentrations from 1×10^{-5} M to 1×10^{-4} M. Its pair was run simultaneously as a control. (Krebs–Ringer bicarbonate solution was used in all experiments, with the following composition: $CaCl_2$, 2.5 mM; KCl, 3.5 mM; NaCl, 117 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM; $NaHCO_3$, 56 mM; glucose, 11 mM.)

Maximum force of contraction of an isometric twitch for nerve and muscle stimulation was measured by a force transducer throughout the experiment. (Maximum force of contraction was found to be developed when the hemidiaphragm was 0.5 to 1.0 mm longer than resting length. Preparations were stimulated with a square wave pulse at a frequency of 12 c.p.m. and a duration of 0.2 ms.)

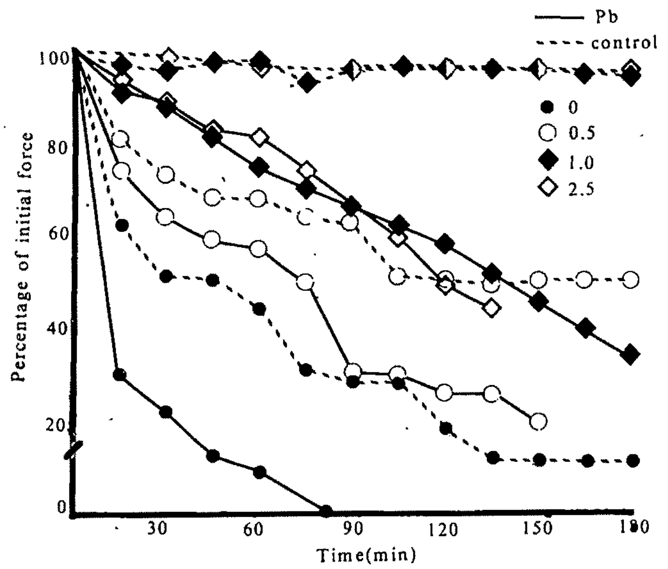

Fig. 1 Change in force of contraction (as percentage of initial force) over 3 h with varying concentrations of calcium, in lead-treated (8×10^{-5} M Pb) and control preparations. Points are means of three experiments.

All experiments were terminated after 4 h. Control preparations showed no significant decline in force of contraction nor significant changes in resting tension for the duration of the experiments.

Table 1 Effect of Lead on the Force of Contraction on Nerve and Muscle Stimulation after Treatment for 2 h

Concentration of lead ($\times10^{-5}$ M)	n	Percentage of initial force of contraction on stimulation through: Nerve	Muscle
0	15	97.5±2.5*	98.0±2.0
1	4	97.5±2.5	98.0±2.0
5	3	77.8±9.7	97.5±2.5
8	6	60.8±5.4	97.5±2.5
10	1	47.7	96.5

* Mean±s.e.m.

Preparations exposed to lead for 2 h showed significant decreases in the force of contraction when stimulated through the nerve (Table 1). The results are expressed as percentage of the initial force of contraction for nerve and muscle stimulation of each preparation. There was no decrease in the force of contraction in controls or in preparations treated with 1×10^{-5} M lead. At 5×10^{-5} M lead, however, the force of contraction on nerve stimulation was reduced significantly to 78% of the initial force (Student's t test[6]). When the same preparations were stimulated through the muscle, no significant decreases in force of contraction were seen at concentrations of lead up to 1×10^{-4} M.

In a separate series of experiments, calcium was added to for preparations after exposure for 2 h to 8×10^{-5} M lead. All preparations demonstrated a decrease in nerve-stimulated force of contraction to a mean of 58% of the initial force. Calcium, when added to produce a final concentration of 3×10^{-3} M, restored the force of contraction to a mean of 91% of initial force for 28 min.

A second series of experiments was done to determine the effect of the concentration of calcium in the bathing medium on the time required for lead to affect nerve-stimulated force of contraction. The concentration of calcium was varied from 0 to 2.5 mM (isotonicity was kept constant by appropriate adjustments in the concentration of NaCl). Isometric twitch tension was measured over 3 h for controls and preparations treated with 8×10^{-5} M lead. Increasing the concentration of calcium from 0 to 1 mM significantly increases the time for equivalent impairment of nerve-stimulated force of lead-treated preparations (Fig. 1). That is, for a given time of exposure to lead, the extent of impairment was inversely related to the calcium concentration. At calcium concentrations above 1 mM, however, no further protection against lead treatment was found.

The motor endplate was tested for possible site of lead action by the addition of exogenous acetylcholine in a range of concentrations from 1×10^{-7} to 5×10^{-6} M to the preparations after treatment for 2 h with 8×10^{-5} M lead. The contraction produced by equimolar additions of acetylcholine was identical in control and lead-treated preparations. These results tend to eliminate the motor endplate as a possible site of the primary toxic actions of lead.

The calcium experiments demonstrated that additions of calcium to normal Krebs–Ringer solution reversed the effects of treatment for 2 h with lead. Additional experiments demonstrated that the time course of impairment of neuromuscular function by lead was related to the concentration of calcium in the bathing media of the preparations. An apparent discrepancy arises between the protective and the reversing effects of calcium. Increasing the concentration of calcium from 1 to 2.5 mM did not significantly change the time course of impairment by lead. In lead-poisoned preparations, however, the addition of 0.5 mM calcium after treatment for 2 h caused a significant recovery of neuromuscular function for 28 min. The timing of calcium addition may be important in producing this reversal. These experiments indicate that calcium and lead may interact in some manner at a site important in neuromuscular function.

The experiments demonstrated that lead, in concentrations greater than 1×10^{-5} M, significantly decreased the force of contraction of the rat hemidiaphragm muscle on phrenic nerve stimulation. Over the same range of concentrations, force of contraction was not affected when the muscle was stimulated directly. Since muscle was not directly involved in the lead toxicity seen in these experiments, the effects of calcium are probably confined to junctional sites. This suggests that lead interferes with either the release of acetylcholine or the uptake of choline and subsequent synthesis of acetylcholine, processes known to have a requirement for calcium (ref. 7 and C. Pert and S. H. Snyder, personal communication).

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Absence of Significant Chromosome Damage in Males Occupationally Exposed to Lead

THE hazard to man of environmental contamination by certain metals is of considerable importance and in this context the organic and inorganic compounds of lead are the subject of much current interest. The clinical effects on the individual of accidental overexposure to lead are well known. What is perhaps not so widely realised, however, is that there is the possibility that high lead levels may be hazardous to the foetus of exposed parents. "Intoxication with lead" is often quoted as "reducing fertility and increasing the frequency of abortions and miscarriages" in man[1–5], and some animal studies have shown[6] that administration of lead salts may result in an increase in congenitally malformed offspring. These findings, the reported carcinogenicity of lead salts in rats[7], and the earlier reports that salts of a variety of heavy metals induce aberrations in plant chromosomes[8,9], have stimulated a number of *in vitro* and *in vivo* studies into the possible mutagenic effects of lead which have yielded conflicting results.

Schwanitz *et al.*[10] reported a three- to four-fold increase in chromosome aberration frequency in human lymphocytes treated *in vitro* with lead acetate as compared with control cells exposed to sodium acetate. In contrast, an extensive *in vitro* study on cultured Chinese hamster fibroblasts by Bauchinger and Schmid[11] showed no difference between control (sodium acetate) and lead acetate treated cells.

In vivo studies on mice fed on a diet containing lead acetate were reported to reveal an increased incidence of chromatid damage in cultured bone marrow cells from treated as compared with control animals. But other investigations[12] on the effect of tetraethyl lead and lead acetate in the mouse showed that neither compound, even at the maximum tolerated dose levels, promoted a dominant lethal response. In man, an increase in chromosome aberrations, particularly chromatid breaks and gaps, was reported by Schwanitz *et al.*[10] to occur in the peripheral blood lymphocytes of eight male workers in a lead oxide factory.

In view of these conflicting reports our study was set up to examine the extent of chromosome damage, and any correlations between the level of damage and the lead levels in blood and urine, in a population of men occupationally exposed to oxides of lead.

The population, described in greater detail in the accompanying paper[13], consisted of seventy male workers employed by a shipbreaking yard. Thirty-five men (Group 1) were engaged as burners to cut the hulls and all metal structures of ships, and their lengths of exposure ranged from 3 months to 43 yr. Their age range was 21–63 yr and the average weekly exposure time was 50 h. The remaining thirty-five men were of comparable age, were not actively engaged in burning, and formed a control group (Group 2).

All the men were medically examined and a full medical and occupational history taken[13]. Each worker had a periodic mass chest X ray and many had also received further radiation exposure for investigations of bone fractures and stomach ulcers. None of the men had undergone radiotherapy, and none were receiving drugs considered to produce chromosome damage. Blood samples from the men were analysed for lead content and routine haematology, and urine samples for lead and coproporphyrins[13].

The sample of blood obtained from each individual for chromosome analysis was coded by a person not concerned in the study, before being set up in culture. The lymphocytes were cultured in Hams F10 medium with 14% bovine serum and colcemid added for the last 3 h of culture. For slide preparation, the cells were subjected to a brief hypotonic treatment with KCl[14] before fixation in 3:1 (methanol-acetic acid) and preparations were made by air drying. To minimise any culture artefacts and to ensure that the majority of the dividing lymphocytes were examined at their first *in vitro* metaphase, the total culture time was either 45 or 48 h. One hundred mitoses with forty-five centromeres or more from each individual were studied by one person. The first two cells were fully analysed to ascertain the chromosome constitution of the individual and all cells were scored for aneuploidy and chromatid and chromosome aberrations.

The blood of sixty-six of the seventy workers was cultured successfully but the cultures from the four remaining individuals, all of whom were in Group 2, yielded few mitoses of good quality and were not scored for aberrations. One of these men was found to have a 47,XYY chromosome constitution, but the remainder had normal male karyotypes.

After the scoring was completed, the slides were decoded and the cytological results obtained from the burners (Group 1) compared with those from the intended controls (Group 2). At the same time, the results from the toxicological studies were made available and from these it was apparent that a large proportion of men in the intended control group had elevated lead levels in blood and urine at the time of sampling due, presumably, to their working in the vicinity of the burners. The cytogenetic results summarised in Table 1 have therefore been divided into the four classes, A to D, based on lead levels in blood[13], and further subdivided into results obtained on individuals in Groups 1 and 2 within each class.

Analysis of the data presented in the form shown in Table 1 did not reveal any consistent and significant differences between the incidence of chromosome-type and chromatid-type aberrations between Groups 1 and 2 or between classes A, B, C and D. Moreover, the results within any class were similar to those obtained from previous surveys of adult males from a variety of groups that were not considered to be unduly exposed to known or suspected mutagens (Table 1).

In considering the comparisons made between groups having different blood lead concentrations, it should be remembered that both blood and urine samples for lead estimations were obtained at the same time as blood for culture, so that these lead estimates relate only to the levels in those individuals on that particular day. The lead level in blood depends on the degree of exposure over the few days before sampling and may therefore vary considerably. On the other hand, chromosome damage in human peripheral leukocytes can reflect damage sustained many years before blood sampling (for example, in individuals exposed to ionising radiations, see below). For this reason, although we had not found any significant differences between the different blood lead-level classes, or between Groups 1 and 2, further comparisons were made within the Group 1 population. Attention was paid to the duration of exposure (number of years worked in the yard and any previous occupational history) and also to the presence of any physical or clinical (including haematological) symptoms which, however mild, could be indicative of lead poisoning. These analyses did not reveal any bias of the distribution of aberrations within the subgroups.

Although the data from the workers exposed to lead fume showed very small increases in the frequencies of chromatid breaks, and in the total number of cells with chromosome-type aberrations, the aberration frequencies were very low and were not significantly different from either control population, despite the fact that the burners, as a group, had possibly received more diagnostic X-ray exposure. There was, however, a significant difference (χ^2 (1 degree of freedom) = 31.4, $P < 0.01$) between burners and controls from the general population (but not shipyard controls) in the total number of cells which had chromatid-type aberrations. This was due to an increased incidence of chromatid and isochromatid gaps and chromatid breaks in the Group 1 men (Table 1). The scoring of these particular aberrations is extremely subjective, and whereas the cells from the Group 1 individuals were scored by a single observer, the figure derived from the 'general population' is a combination of results from several observers. These parameters of chromosome damage may be unreliable indicators due to the difficulties of distinguishing achromatic non-stained regions (gaps) and of confusing them with chromatid and isochromatid discontinuities (breaks). These can only be reliably distinguished by observing the cells at metaphase and anaphase and thus following the types of metaphase aberrations which produce fragments at anaphase. We must therefore conclude that our results do not point to an increased chromosome aberration frequency in the peripheral blood lymphocytes of individuals chronically exposed to high levels of lead oxides.

While our study was in progress, Bauchinger and Schmid[15] reported on results obtained on lymphocyte chromosomes of twenty industrial workers who had been exposed for several years to lead dust, and on twenty-nine policemen "employed in the Munich areas with the highest traffic density" and who showed a 20–30% increase in blood lead levels as compared with the average population. In this work cells were cultured for 46 h and in neither of these groups was there any increase in aberration yield levels over controls.

In recording our negative findings, it is important to ask what value can be placed on the earlier reported positive result of Schwanitz *et al.*[10]. In that study no information is given on the duration of culture and on whether blood cultures from control and exposed individuals were set up in the same way and at the same time. The authors recorded approximately twice as many mitoses in cultures from exposed as from control individuals and if, as seems probable, the cells from the exposed individuals were cultured for 3 d, then a large proportion of the metaphase cells may have been in their second or later mitosis in culture[16,17]. It is well known, from studies on normal and irradiated human blood cells, that the incidence of gaps and chromatid aberrations in lymphocyte chromosomes is increased if cells are allowed to proceed through two or more cycles in culture, as also is the incidence of polyploid mitotic figures and of "derived" chromosome-type changes[18]. The fact that Schwanitz *et al.*[10] observed some eighteen tetraploid metaphases in a total of 800 scored in the exposed individuals (compare the 0 in 3,500 scored in the present work) and that the excess aberrations in this group were "secondary aberrations", that is, largely chromatid and isochromatid gaps, suggests that most of these aberrations were not present in these cells *in vivo*, but developed during culture. This suggestion raises the very important general question of interpretation in the case of chromosome damage in lymphocytes obtained from individuals who may or may not have been exposed to an environmental mutagen.

Many of the lymphocytes in human peripheral blood are long lived and a significant proportion rarely undergo mitosis *in vivo*[19]. Chromosome damage sustained by these cells at the time of their exposure to a mutagen may therefore be detectable for many years after exposure, as in the case of the Japanese A-bomb survivors who show high aberration yields some 25 yr after their original exposure[20]. Circulating lymphocytes are in a G_1 state so that exposure to ionising radiations induces chromosome-type changes where the unit of breakage and exchange is the whole chromosome giving the characteristic dicentrics, rings and chromosome fragments. Irradiation of the S or G_2 phases of the cell cycle gives chromatid-type aberrations where the unit of breakage or exchange is the single chromatid. In contrast with ionising radiations, many chemicals produce only chromatid-type aberrations in the first and subsequent mitosis following exposure, even though the cells may have been exposed to the mutagen while in the G_1 phase of the cycle[18]. The less frequent chromosome-type changes seen in second, or later, divisions following treatment with these chemical mutagens result from the duplication of chromatid aberrations that were transmitted to daughter cells, and are thus 'derived' chromosome changes[18].

In the case of chronic *in vivo* exposure to a chemical mutagen, one might expect to observe both chromatid and derived chromosome damage in lymphocytes at their first mitosis in culture. The incidence of such damage will, however, be dependent not only on the accumulated dose, but also on the interval between exposure and time of sampling for culture. With increasing time there would be an increasing probability of obtaining derived chromosome changes due to cell turnover *in vivo*, but a corresponding reduction in the incidence of chromatid-type changes due to cell, and aberration, loss and possibly also to repair of lesions in G_1 chromosomes. Chromatid damage observed at the first mitosis in culture will therefore be heavily influenced by the amount of mutagen present in the body over a relatively short time before sampling and also by the amount of mutagen inevitably carried over in the blood into the culture medium.

Table 1 Cytogenetic Findings in Cultured Lymphocytes from Shipyard Workers with an Occupational History of Lead Exposure (Group 1), and from Shipyard Controls (Group 2) and Other Adult Males

					Cells with chromatid aberrations					Cells with chromosome aberrations				
Category	Blood lead level (μg per 100 ml)	No. of men studied	Total cells scored	% Normal cells	Total No.	%	Chromatid and and isochromatid gaps	Chromatid breaks	Chromatid interchanges	Total No.	%	Dicentrics	Acentric fragments	No. structurally abnormal chromosomes*
A. Normal	<40	12 (Group 2)	1,200	91.5	51	4.25	53	0	0	5	0.42	2	5	0
B. Acceptable	40–80	16 (Group 1)	1,600	93.33	71	4.43	68	7	2	7	0.44	1	6	5
		17 (Group 2)	1,700	94.50	70	4.12	73	1	2	6	0.35	0	5	2
		33 (total)	3,300	93.47	141	4.27	141	8	4	13	0.39	1	11	7
C. Excessive	80–120	15 (Group 1)	1,500	93.87	72	4.80	68	5	0	13	0.87	0	7	3
		2 (Group 2)	200	94.29	10	5.00	10	1	0	1	0.50	0	1	0
		17 (total)	1,700	94.09	82	4.90	78	6	0	14	0.82	0	8	3
D. Dangerous	>120	4 (Group 1)	400	94.50	25	6.25	25	0	0	3	0.75	0	3	0
Total Group 1 (Exposed)	—	35	3,500	93.90	168	5.16	161	12	2	23	0.69	1	16	8
Total Group 2 (Control)	—	31	3,100	93.43	131	4.46	136	2	2	12	0.42	2	11	2
Other males†	—	285	3,107	96.66‡	68	2.18	65	4	0	36	1.16	2	9	16

* Number of structurally abnormal monocentric chromosomes observed which were not associated with an acentric fragment.

† Data obtained from previous MRC surveys of other adult male groups with no history of undue exposure to known or suspected mutagens (lymphocyte culture time <50 h).

‡ Includes non-modal cells.

In terms of potential genetic hazard to a population, the production of a low level of damage in a large number of individuals is of greater consequence to the population than the induction of large amounts of damage in a small number of individuals. The human peripheral blood lymphocyte offers a convenient means of detecting low levels of chromosome damage which might be caused by an environmental agent and may provide a useful screening system. But, apart from the considerable technical labour involved, there may be difficulties both with the interpretation of chromosome aberration data and with the extrapolation of results from somatic to germ cells. Problems of interpretation are further complicated by mutagens which produce chromatid-type aberrations, since similar changes may also develop in cultured cells as a consequence of infection or of non-specific 'adverse culture conditions'. In such work it is obviously of paramount importance to use optimum culture conditions and to obtain proper control specimens matched not only in terms of donor characteristics but also with regard to culture conditions.

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Lead Over-absorption in a Population of Oxy-gas Burners

Pliny (AD 23-79) observed that in the second century BC the ancients painted their ships with native ceruse (white lead), that lead poisoning was known in his day and that the workers in lead products tied up their faces in loose bags lest they should inhale the pernicious dust[1]. In a survey of 228 men in nine shipbreaking yards in Scotland and NE England, McCallum[2] focused attention on the health hazard resulting from burning lead-painted metal with a high temperature flame. He found 36% of the burners with a haemoglobin content less than 12.5 g per 100 ml of whole blood. In this operation, in which oxy-gas torches have been used at the work face, temperatures are in the region of 3,000–3,500° C. Iron melts at 1,535° C and is found in vapour form at 3,000° C. Lead melts at 328° C and boils at 1,740° C. This produces a risk of excessive exposure in compartments and between decks of ships, of which lead from paintwork is the principal risk[3]. In the breathing zone of the burner, with maximum concentrations of fume at torch level, may also be found zinc (zinc metal fume fever), copper from piping and cadmium from protective coatings[4].

The limitation of statutory control measures and the difficulty of clinically detecting lead absorption (as opposed to lead poisoning) have led to ineffective control in shipbreaking processes compared with other industries with a known lead risk (as, for example, in lead accumulator manufacture). The evaluation of the degree of lead absorption and the identification of lead overexposure is clearly the right objective in modern conditions, rather than the identification of lead poisoning[5]. It is not yet known what levels of lead absorption are tolerable but Lane *et al.*[6] have proposed four arbitrary categories, (A) absorption found in the 'normal' population (blood lead <40 μg per 100 ml; urinary lead <80 μg l^{-1}); (B) increased but acceptable absorption (blood lead 40–80 μg per 100 ml; urinary lead 80–150 μg l^{-1}); (C) excessive and unacceptable absorption (blood lead 80–120 μg per 100 ml; urinary lead 150–250 μg l^{-1}); and (D) dangerous absorption, suspend automatically from work with lead (blood lead >120 μg per 100 ml; urinary lead >250 μg l^{-1}).

This paper is concerned with the examination of a population of oxy-gas burners in a shipbreaking yard exposed to lead fume in their work environment. In the accompanying paper[7] cytogenetic studies on this population are described.

In one shipbreaking yard thirty-five males engaged on oxy-gas burning (Group 1) and a control group of thirty-five men of comparable age employed as labourers, crane drivers, and so on (Group 2) were investigated. The age distribution of the population and the length of time the oxy-gas burners had been actively burning are shown in Tables 1 and 2.

Table 1 Ages of Burners and Controls in Ten-yr Age Groups

Age (yr)	No. of burners, ship and shore (Group 1)	No. of controls (Group 2)
15–24	6	6
25–34	11	3
35–44	2	9
45–54	8	9
55–64	8	8
Total	35 (Mean age 41 yr)	35 (Mean age 43 yr)

Table 2 Length of Time Employees (Group 1) had been Burners

Oxy-gas burning (yr)	Number of Men	%
Up to 5 yr	20	57
5 yr and over	15*	43
Mean 8.8	35	100

* Two of these men had 42 and 43 yr continuous burning.

A full occupational and medical history, including previous spells of burning at other yards, was obtained for burners and controls, with emphasis on the symptoms of lead absorption, which are colic, dyspepsia, lassitude and loss of appetite, constipation, muscle aches and headache. The physical signs of lead over-absorption, such as paresis, pallor and blue line on the gums, were then looked for. In the first survey a sample (20 ml) of venous blood was taken from each man and divided into two equal parts, one being used for lymphocyte culture for chromosome analysis and the other for blood lead content and haematological studies. In addition, urinary lead determinations were carried out by the Central Reference Laboratory of the Department of Employment. In order to obtain a more accurate picture of the extent of lead absorption of this population previous to and at the time of the cytogenetic study, four additional burner population surveys for blood and urine lead were made at three-monthly intervals. An environmental survey was carried out by the Industrial Hygiene Laboratory of HM Factory Inspectorate. Samples were taken from the breathing zone of burners cutting heavily painted panels of a merchant ship and of a naval frigate.

The mean figures obtained for the burners and controls from the first survey for lead levels in blood and urine are shown in Table 3, together with the positive findings of the clinical examination. It should be noted that the thirty-five burners sampled gave a mean haemoglobin value of 14.8 g per 100 ml with seven under 14.0 g and one under 12.5 g per 100 ml.

Table 3 Survey 1. Lead Levels of Blood and Urine, and Clinical Symptoms

Population	Blood lead (μg per 100 ml)	Urine lead (μg l^{-1})	Colic	Constipation	Pallor	Lassitude	Blue line
Group 1*	mean=91.0 (35 men)	median=144 (32 men)	4	3	4	4	5
Group 2†	mean=49.7 (34 men)	median=24 (32 men)	–	–	–	–	–

* Oxy-gas burners.
† Controls.

The difference in mean blood lead levels between Group 1 and the Group 2 controls is significant at the 0.1% level. A number of urine lead estimations were below the lower limit of detection of the chemical method, so that the urine lead results were analysed by non-parametric tests where medians were used instead of means. The difference between the oxy-gas burners and the control population was once again significant at the 0.1% level. The detailed data, together with the results from subsequent surveys on the burner population, are summarised according to the classification of Lane *et al.*[6] in Tables 4 and 5.

Table 4 Lead Absorption Categories[6] in Blood in the Four Surveys of a Population of Oxy-gas Burners

Survey	Date	Absorption category (μg per 100 ml) A Normal (<40)	B Acceptable (40–80)	C Excessive (80–120)	D Dangerous (>120)	Total No. of burners
1	November 1971	14	19	2	0	35 controls
1	November 1971	0	16	15	4	35
2	April 1972	0	9	17	12	38
3	July 1972	3	24	7	0	34
4	November 1972	12	24	2	0	38

The results of the environmental survey showed that from heavily painted plates of a merchant ship and hull sections of a warship, concentrations approaching 4.0 mg and 14.0 mg m^{-3} lead-in-air respectively were produced in the breathing zones. These values are very much higher than the threshold limit value for a 40 h week of 0.2 mg m^{-3} (ref. 8). There is, however, no accurate method of computing actual lead intake from concentrations of lead-in-air which vary over very wide limits dependent on the presence or absence of lead paint, constantly varying air movements, and variable absorption due to voluntary use of respirators.

Table 5 Lead Absorption Categories[6] in Urine in the Four Surveys of a Population of Oxy-gas Burners

Survey	Date	Absorption category (μg l^{-1}) A Normal (<80)	B Acceptable (80–150)	C Excessive (150–250)	D Dangerous (>250)	Total No. of burners
1	November 1971	28	5	0	0	33* controls
1	November 1971	6	11	10	7	34*
2	April 1972	10	16	6	6	38
3	July 1972	15	15	4	0	34
4	November 1972	23	12	3	0	38

* Excluding samples that were contaminated and not recorded.

The results of biological tests on the thirty-five oxy-gas burners in this study confirm the views of McCallum[2,3]. In our initial survey, high blood lead levels were found in some of the selected controls, but all individuals in Group 1 had more than 40 μg of lead per 100 ml of blood, with four men in the dangerous (D) category (Table 4). In the second survey carried out three months later, a greater proportion of the exposed population had higher blood lead levels, with twelve men in the D category. These burners with the highest blood lead levels were then removed from burning where there was any risk of lead in the fume and subsequent surveys (Table 4) showed a marked reduction in blood lead levels. The urine lead values (Table 5), while not correlating with the blood values, showed the same degree of improvement over one year's monitoring. The data obtained in these surveys show that by close supervision of the work situation, it is possible to achieve a degree of lead absorption which is acceptable by today's arbitrary standards.

A number of questions remain unanswered in the present shipbreaking work environment. One is the permanence or long-term medical effects of lead absorption in which Rieke[9] has recently expressed concern, particularly with regard to the detection of so-called 'susceptible' burners. A second is the possibility of chromosome damage and this is the subject of the accompanying paper[7].

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Clonal Restriction of the Immune Response to Phosphorylcholine

THE antibody response to most antigens is chemically heterogeneous, reflecting clonal heterogeneity of the responding cells. This heterogeneity may arise in part from the number of different epitopes present in the structure of complex antigens, although the antibody response even to chemically simple haptens (which may contain more than one epitope) is often very heterogeneous. For example, the dinitrophenyl hapten on a protein carrier elicits many different anti-hapten antibodies which are distinguished by isoelectric focusing[1]. Also, individual mice respond to the *p*-azobenzoate and to the *p*-azophenylarsonate haptens by producing a population of serum antibodies with many idiotypic specificities[2]. Certain carbohydrate antigens, however, seem to induce relatively homogeneous antibody responses[3,4]. One of these antigens, the C-polysaccharide from the rough strain of pneumococcus R36A, induces a restricted antibody response directed against phosphorylcholine[5], a structural component of the C-polysaccharide[6].

We have examined the antibody response of Balb/c mice to phosphorylcholine by testing various inhibitors for their capacity to interfere specifically with antibody activity. These inhibitors include (*a*) phosphorylcholine and phosphorylcholine analogues to determine the epitopic specificity of the antibodies and (*b*) antisera raised against myeloma proteins which have antibody activity against phosphorylcholine. These anti-idiotypic sera provide a measure of the idiotypic, and thus clonal homogeneity of the response. By these methods we find that the antibody response is remarkably homogeneous and has the characteristics of a so-called "monoclonal" response. In fact, the antibody response is equally homogeneous whether immunisation is with the C-polysaccharide or with phosphorylcholine coupled to various protein carriers. Furthermore, when this response is suppressed, other clones of cells producing antibody to phosphorylcholine do not appear.

p-Diazophenylphosphorylcholine was synthesised according to the method of Chesebro and Metzger[7]. This compound, in *p*H 9.2 borate buffer, reacts with tyrosyl residues of proteins to yield the conjugated hapten, *p*-azophenylphosphorylcholine (PC). Balb/c mice were immunised with PC conjugated to keyhole limpet haemocyanin (PC-KLH) or PC conjugated to *Salmonella* flagella (PC-FLA). The synthesis of antibodies by individual spleen cells was demonstrated by the haemolytic plaque technique, using as target cells sheep erythrocytes conjugated with PC (PC-SRBC)[5].

Table 1 Response to *p*-Azophenylphosphorylcholine Conjugated to Different Carriers

Antigen	Carrier priming	PFC per spleen $\times 10^3$
10^9 cells R36A	—	78.4± 6.3
200 μg FLA	—	2.3± 1.2
200 μg PC-FLA	—	287.3±27.0
20 μg PC-FLA	—	162.0± 7.2
200 μg KLH	—	0.9± 0.2
200 μg PC-KLH	—	151.0±18.0
20 μg PC-KLH	—	95.0±13.0
200 μg PC-FLA	5 μg FLA	269.0±20.8
20 μg PC-FLA	5 μg FLA	148.3±16.0
200 μg KLH	5 μg KLH	1.8± 0.6
200 μg PC-KLH	5 μg KLH	439.8±52.4
20 μg PC-KLH	5 μg KLH	328.4±24.3

8–12-week-old Balb/c mice were immunised intravenously with antigen 4 d before being assayed for their splenic PFC response. PC-KLH and PC-FLA were in saline solution while the R36A vaccine was a saline suspension of the formalin-killed bacteria[5].

The magnitude of the IgM plaque-forming cell response is given in Table 1; about 8×10^4 plaque-forming cells (PFC) per spleen were detected in mice immunised with the R36A pneumococcal vaccine while immunisation with either 200 μg of PC-KLH or of PC-FLA induced about 15×10^4 and 30×10^4 PFC per spleen respectively. Immunisation with either carrier alone caused an insignificant response to PC. Thus, the hapten on either carrier elicited a splenic PFC response slightly greater than that induced by the vaccine. When mice were primed with a small dose of KLH 3 d before immunisation with PC-KLH, the anti-hapten response was about three times greater than the response obtained from unprimed mice. No such carrier priming effect was observed with the PC-FLA antigen.

While the effect of carrier priming was demonstrable for PC-KLH, the effect of priming is even more pronounced for other carriers. For example, PC coupled to bovine gamma globulin (BGG) induces a very low response in Balb/c mice; however, the response is comparable with that reported here when mice are primed with BGG (W. L., unpublished results). Thus, carrier-specific cells are required, at least for some PC-carrier conjugates; and, by inference, the magnitude of responses to these conjugates is a function of the number of carrier-specific cells present.

Indirect plaques were not detected during the primary or the secondary response to PC induced by immunisation with either PC-KLH or PC-FLA. The developing serum used for enhancement of IgG PFC was a goat anti-mouse IgG serum which was effective for demonstrating indirect plaques against sheep red blood cells (SRBC)[8]. Apparently, the response is restricted to production of IgM antibodies.

The specificity of the PFC response to PC was studied using analogues of *p*-azophenylphosphorylcholine to inhibit plaque formation by spleen cells from mice immunised with different PC-carrier conjugates; 10^{-2} mg ml^{-1} PC-BSA or 10^{-2} M phosphorylcholine solutions inhibited formation of plaques. In contrast, choline alone, acetylcholine alone or 2-dimethylaminoethylphosphate alone, at the same molarity, failed to inhibit plaque formation, Table 2*a*. Thus, it seems that the intact zwitterionic phosphorylcholine molecule is required to inhibit the binding of anti-hapten antibodies to the PC-SRBC. None of the above compounds inhibited plaque formation by spleen cells from mice immunised with

Table 2 Inhibition of Plaque Formation

Inhibitors	% Inhibition of anti-hapten plaques from mice immunised with			
	PC-FLA	PC-KLH	PC-KLH (KLH-primed)	TNP-KLH
(*a*) Hapten analogues				
—	0±3%	0±4%	0±2%	0±4%
10^{-2} M phosphorylcholine	98±2%	98±3%	99±2%	4±3%
10^{-2} mg ml^{-1} PC-BSA	97±2%	97±2%	97±3%	2±4%
10^{-2} M choline	0±2%	2±4%	4±3%	0±4%
10^{-2} M acetylcholine	1±4%	1±4%	4±2%	0±4%
10^{-2} M 2-dimethylaminoethyl phosphate	1±4%	3±3%	2±3%	2±7%
10^{-2} mg ml^{-1} BSA	−6+5%	−2±4%	0±4%	2±5%
(*b*) Anti-idiotypic sera				
anti-TEPC-15	97±3%	99±3%	96±2%	−2±4%
anti-MOPC-167	−2±3%	−7±5%	0±4%	−5±4%
anti-McPC-603	5±3%	4±4%	7±4%	2±4%

The anti-hapten PFC were from Balb/c mice immunised intravenously with antigen 4 d before assay. Inhibition of plaque formation was carried out as described previously[5]. The effect of each inhibitor was assayed in triplicate. Inhibition is presented as the % inhibition of plaques±s.e. by each agent. Anti-TNP plaques were used to test for the non-specific inhibitory activity of the agents used. Anti-TNP PFC were assayed using TNP-SRBC as target cells[9]. The anti-idiotypic sera used for plaque inhibition were absorbed with normal Balb/c serum, heat-inactivated and diluted 1 : 100 in Hanks balanced saline solution before use in this assay. The concentration of phosphorylcholine required for 50% inhibition is the same for PFC from PC-FLA, PC-KLH or R36A immunisation. Furthermore, the inhibition curve for plaques from animals immunised with PC-KLH indicates homogeneity of binding affinities among the PFC (W. L., unpublished results).

the trinitrophenyl hapten conjugated to KLH (TNP-KLH) and assayed against TNP-SRBC[9]. From these findings, we conclude that the anti-PC response measured by this assay is directed to phosphorylcholine itself and not to determinants introduced by the conjugation of phosphorylcholine to carriers.

The idiotypic homogeneity of the response to the PC hapten was studied by the plaque inhibition technique[5] using antisera raised against the myeloma proteins TEPC-15, MOPC-167 and McPC-603. Although these Balb/c myeloma proteins bind phosphorylcholine, each possesses a different idiotype[10]. The antisera, raised in A/He mice[5,10] to these three proteins, were used to determine the idiotypic specificity of the PFC response to PC, Table 2*b*. Anti-MOPC-167 and anti-McPC-603 had no inhibitory effect while anti-TEPC-15 inhibited plaque formation by cells from mice immunised with either of the PC-carrier conjugates. None of the antisera inhibited plaque formation to the TNP hapten. Thus, the response to phosphorylcholine is of an idiotype identical to or cross reactive with the idiotype of the TEPC-15 myeloma protein.

Mice given anti-TEPC-15 serum before immunisation have reduced responses to phosphorylcholine[11]. Suppression is due to inhibition of immunisation rather than to inhibition of plaque formation, since no suppression occurs when the antiserum is given 1 d after immunisation[11]. In our experiments, anti-TEPC-15 sera were administered to Balb/c mice 1 d before immunisation with PC-carrier, Table 3. The response of these mice, after immunisation with PC-FLA or PC-KLH, was less than 5% of the response of normal mice immunised with the same antigen. The idiotypic specificity of suppression was demonstrated by the failure of anti-MOPC-167 or of anti-McPC-603 to suppress the responses induced by PC-KLH. That the suppression was specific for the PC hapten was shown by the inability of anti-TEPC-15 serum to diminish the response to the TNP hapten. In fact, when mice were immunised with both PC and TNP conjugated to the same carrier molecule (PC-KLH-TNP), anti-TEPC-15 serum suppressed only the response to PC, Table 3; obviously carrier-specific helper cell activity for TNP is not affected by suppression of the response to PC.

Table 3 Specific Suppression of the Response to Phosphorylcholine by Anti-idiotypic Serum

Antigen	Antiserum	PFC per spleen × 10^3 PC	TNP
50 µg PC-FLA	—	112.9 ± 12.1	
50 µg PC-FLA	anti-TEPC-15	1.6 ± 0.9	
200 µg PC-KLH	—	140.5 ± 11.7	
200 µg PC-KLH	anti-TEPC-15	3.1 ± 1.3	
200 µg PC-KLH	anti-MOPC-167	136.3 ± 8.2	
200 µg PC-KLH	anti-McPC-603	154.2 ± 13.5	
200 µg TNP-KLH	—		283.9 ± 15.4
200 µg TNP-KLH	anti-TEPC-15		294.0 ± 23.9
200 µg PC-KLH-TNP	—	99.6 ± 12.9	120.3 ± 4.0
200 µg PC-KLH-TNP	anti-TEPC-15	1.9 ± 1.3	106.8 ± 10.3

Balb/c mice treated with anti-idiotypic sera were given 0.3 ml of the appropriate antisera intravenously 1 d before immunisation with antigen, and their spleens assayed for anti-hapten PFC 4 d after immunisation. Anti-PC PFC were assayed using PC-SRBC as described in Table 1. Anti-TNP PFC were assayed using TNP-SRBC[9]. The anti-hapten PFC per spleen are the mean from groups of three mice ± s.e.m.

Whereas suppression of induction of the response to PC by antibody to TEPC-15 is considerable, it is not complete. The PFC still detectable in suppressed mice were analysed to determine whether the antibodies secreted by these cells were directed to phosphorylcholine and if so whether they bear the TEPC-15 idiotype. The results, Table 4, indicate that about 50% of these residual plaques are no longer inhibited by free phosphorylcholine. Of the plaques that are inhibitable by phosphorylcholine, apparently very few, if any, are of the TEPC-15, MOPC-167 or McPC-603 idiotypes, since the antisera to these myeloma proteins did not significantly inhibit the residual plaques. Thus, the epitopic specificity and the idiotypic composition of the residual response after suppression with anti-idiotypic serum to TEPC-15 are different from the normal anti-PC response.

We have shown that Balb/c mice respond as well to phosphorylcholine whether they are immunised with the R36A pneumococcal vaccine or with *p*-azophenylphosphorylcholine-carrier conjugates. The inhibition studies demonstrate that the measured response to *p*-azophenylphosphorylcholine is directed to the phosphorylcholine moiety of the hapten. The response to phosphorylcholine is also restricted to the idiotype present in the TEPC-15 myeloma protein regardless of the carrier used to induce the antibody response. Furthermore, priming with carrier does not affect this idiotypic restriction. These findings lead us to conclude that the same B cell clone or clones having receptors for phosphorylcholine are stimulated regardless of the carrier used. Apparently the carrier has no effect on the selection of the B cell clone responding to PC.

Table 4 Analysis of the PFC Remaining after Suppression by Anti-Idiotypic Serum

Inhibitor	% Inhibition of anti-PC plaques from mice immunised with PC-FLA	PC-KLH (KLH-primed)
—	0 ± 5%	0 ± 7%
10^{-2} M phosphorylcholine	54 ± 7%	45 ± 9%
10^{-2} mg ml^{-1} PC-BSA	96 ± 4%	95 ± 6%
anti-TEPC-15	7 ± 4%	6 ± 6%
anti-MOPC-167	−4 ± 7%	−4 ± 8%
anti-McPC-603	13 ± 6%	1 ± 10%

The plaques studied here were from animals suppressed with anti-TEPC-15 and immunised with 200 µg of PC-carrier intravenously 1 d later. PC-KLH-immunised mice had been primed with 5 µg KHL 3 d before immunisation. The assay technique and the statistical analysis were the same as those used for the data in Table 2.

The residual response in suppressed mice is not of the TEPC-15 idiotype. Furthermore, this response may involve the chemical bridge between PC and the carrier, since plaque formation is suppressed by PC-carrier but not by phosphorylcholine alone. Whether this residual response is present as a minute and hence undetectable fraction of the normal response, or whether it arises only after suppression of the dominant TEPC-15 idiotype, remains undetermined.

The possibility existed that, on immunisation of mice with PC, expression of the dominant TEPC-15 idiotype might be responsible for suppressing the expression of other idiotypes. For example, in the heterogeneous responses to haptens such as DNP and *p*-azophenylarsonate, the loss of expression of one idiotype or clone is followed by compensatory activation of other clones, some of which were previously silent[12,13]. When the clone or clones of cells expressing the TEPC-15 idiotype are suppressed, however, other clones fail to expand appreciably, if at all. This makes it unlikely that expression of the dominant TEPC-15 clone is actively suppressing the other clones. Furthermore, mice suppressed with anti-TEPC-15, and then challenged several times with PC-carrier until a PFC response to PC is again detected, still expressed only the TEPC-15 idiotype (unpublished results of W. L.). This recovery from suppression with expression of the suppressed idiotype strongly suggests that the response of Balb/c mice to PC is restricted to the TEPC-15 idiotype. Presumably, this restriction in the adult Balb/c mouse is due either to clonal selection during ontogeny or to the absence or genetic deficiency of other clones. The study of the response to phosphorylcholine in mice which have been idiotypically suppressed at different stages of ontogeny may indicate the origin of this clonal restriction. In addition, the natural occurrence of a response which is restricted to the expression of one dominant idiotype may provide an experimental system for the study of the senescence of a repeatedly stimulated clone in the same animal. These possibilities are under investigation.

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Lacunae in Erythrocyte Monolayers induced by Adherent Leukocytes

POLYMORPHONUCLEAR leukocytes are capable of migration, aggregation, adherence, and phagocytosis as well as the intracellular destruction of bacteria[1]. The observations reported here suggest yet another facility, namely that of repulsion. These experiments indicate that surface adherent leukocytes, whether polymorphonuclear or other types, repel erythrocytes from the immediate zone about them, thus creating lacunae.

Buffy coat fractions were obtained from fresh heparinised human blood by centrifugation in plastic tubing[2]. These were mixed in a vortex shaker with an equal aliquot of red cells diluted ten-fold in 0.9% sodium chloride solution. Ten microlitres of this was added to a siliconised glass slide, covered with an 18 mm circular coverslip, and the edges sealed with paraffin oil. After incubation at 37.5° C for between 30 min and 4 h, the slides were viewed at varying intervals over several days, magnification of 150 to 450, with ordinary phase, interference phase and darkfield phase illumination.

The usual preparation was a uniform monolayer of erythrocytes with a variable number of distinct, round, clear lacunae. In some instances, twin lacunae were seen, and in particularly thin preparations the "lake" pattern could be distorted. These lacunae in the erythrocytes developed early, increased in number and size on standing overnight, and persisted for days. Many were near the edge of the preparation, but they were also seen scattered uniformly throughout. In thick preparations, the lacunae appeared only as light areas in the diffuse redness of multilayered erythrocytes.

Most of the lacunae were acellular except for a single central cell identifiable as a polymorphonuclear leukocyte (Fig. 1), or occasionally as a monocyte, basophil, or lymphocyte. Occasionally, a few platelets or one or two red cells could be seen intruding into the lacuna. A few lacunae were completely devoid of even a central cell. Under phase microscopy[3] many lymphocytes could be identified among the erythrocytes, without any circumcellular space or lacunar formation. Similarly, platelet clumps were seen among the erythrocytes but again without any surrounding clear spaces.

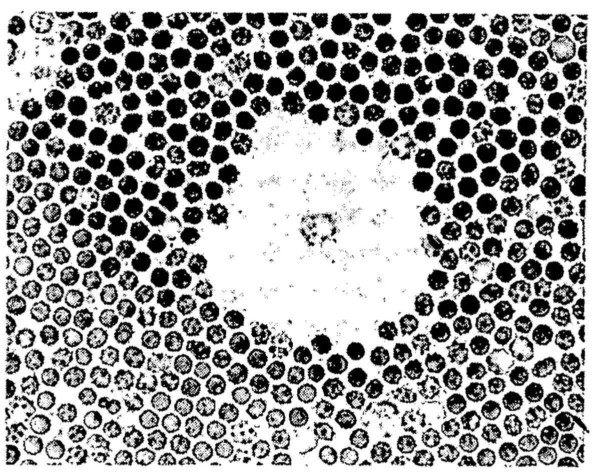

Fig. 1 View of lacuna which appears around an adherent polymorphonuclear leukocyte. In lower part of field, a lymphocyte floats freely in the erythrocytes, failing to show lacuna formation. Unstained, ×419.

The phenomenon of lacuna formation seems not to be limited to human blood, being similarly observed in rabbit, rat and mouse blood. It occurred on plain glass and plastic, as well as on siliconised glass slides, since the serum proteins present seemed to render the surfaces essentially the same[4]. Lacuna formation was also observed in blood contained in a capillary tube. It took longer for the lacunae to appear after incubation at room temperature than at 37.5° C. At 5° C lacuna formation was inhibited, in keeping with the known reduction in leukocyte adherence[5] at this temperature. Both heparinised and EDTA-treated blood were used and the red cells could be suspended successfully in a variety of diluents including Tyrode's and colloidal solutions. Using 1% phosphate buffered saline solutions it was found that acidic preparations were unsatisfactory but those at or above *p*H 7 were satisfactory. Use of borate buffers at *p*H 9 and 10 favoured early formation of lacunae. Red cell dilution was optimal between 1/6 and 1/32, the critical determinant being the achievement of a monocell layer of erythrocytes.

Individual differences in lacuna formation were noted, some of the most rapidly formed lacunae being found in patients with acute infectious disease. The use of vital stains to differentiate the cells did not interfere with lacuna production (Fig. 2*a*, *b*).

It was possible to induce streaming of the ambient red cells by applying gentle pressure on the coverslip. Although this resulted in an elliptical distortion of the lacuna it did not dislodge the central leukocyte, indicating that it is adherent. The significance of this adherence in the formation of lacunae was demonstrated in thick preparations, in which free floating non-adherent leukocytes could be seen unassociated with lacuna formation (Fig. 2*a*), at the same time that deeper focus on the same field (Fig. 2*b*) disclosed a single adherent leukocyte within each lacuna.

The mechanism of lacuna formation remains obscure. Although it can be argued that the weight of the coverslip[6] compresses the leukocyte to balloon dimensions, or that during adherence its hyaloplasm[7] extends to fill the entire lacuna, the cell body as seen with thiazine red R supravital staining, as well as with phase, is significantly smaller than the lacunar space. At first glance, these lacunae also resemble plaques of immune haemolysis[8] but the lacunae we observed are much smaller and phase microscopy reveals

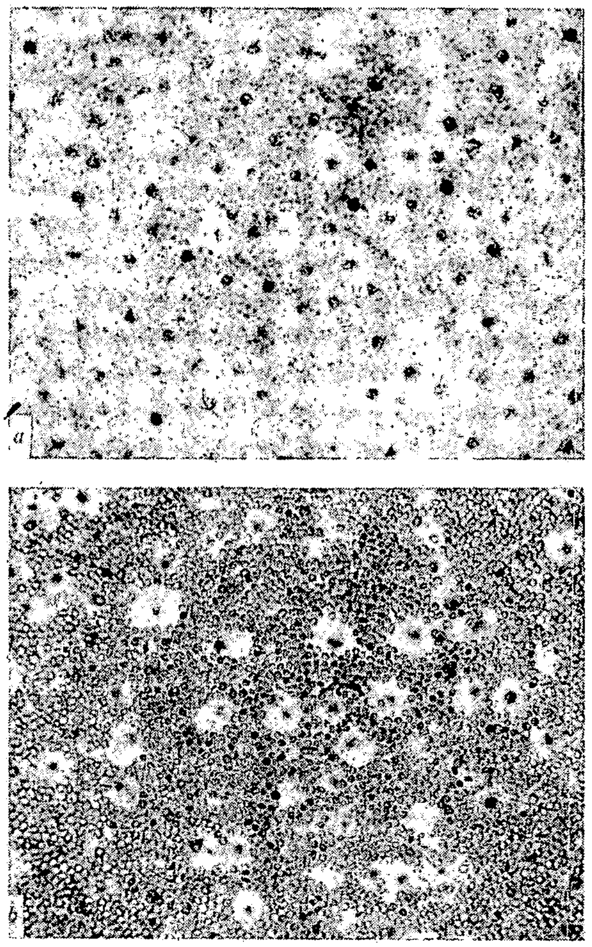

Fig. 2 Lacuna formation with leukocytes stained supravitally with iodonitrotetrazolium. Exactly the same field is seen at two levels. *a*, At higher level non-adherent floating leukocytes are seen with no relation to lacunar spaces. Lacunae are not in focus. ×174. *b*, At deeper level lacunae with central adherent leukocytes can be seen.

no haemolytic ghost cells within them[9]. Further, no foreign mixture of cells was used. Electrostatic forces could be deemed responsible for the erythrocyte repulsion, but changing the charge on the red cell by the addition of formalin or salmine sulphate (10^{-4}) to the diluent had no effect.

It seems more likely to me that the adherent leukocyte secretes a substance which produces a territorial space about it. In support of this is the fact that lacuna formation is accentuated by suspending erythrocytes in a borate buffer at *p*H 10 which could be expected to produce cell irritation. Rosenberg[10] has demonstrated by ellipsometry that the leukocyte can secrete a micro-exudate when in contact with glass, and this material can spread out to several cell diameters. Taylor later visualised such a cell exudate by immunological means[11] and observed that, by virtue of such liberated products, human conjunctival cells inhibit the spreading and contact relations of themselves and nearby cells[12].

In the monocellular layering technique described here, precisely such a leukocyte exudate may be responsible for the lacunae, the absence of erthrocytes indicating a secretion which inhibits their regular distribution and movement. I have been uniformly unsuccessful in staining or visualising any such material in the lacuna. Nonetheless, fixed slides, subsequently washed, show no cells but show clear lacunae by negative contrast when Giemsa stain is used and the slide viewed under phase. Further supporting the view that a secretion could be responsible is the fact that slides with lacunae, washed and resurfaced with red cells, show reformation of lacunae. Finally, it should be pointed out that lacuna formation was not dependent on the presence of erythrocytes, but rather on the adherent leukocyte. Thus, lacunae could be seen when either carbon particles (India ink 1/12), polystyrene latex particles (1.011 μm diameter, 10%, Dow), or colloidal silica (Ludox S 1/30, Dupont) was used in place of erythrocytes as a field indicator.

Further study of lacuna formation seems warranted since these lacunae may provide another source of information of the behaviour of the leukocyte in disease states and in the immune response.

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Is the Area Postrema a Control Centre of Blood Pressure?

SEVERAL antihypertensive drugs have a central site of action[1]. The anatomical structures involved are unknown but there is evidence that some areas in the vicinity of the fourth brain ventricle mediate their effects[1]. Also in the region of the fourth ventricle noradrenaline[2–6] and γ-aminobutyric acid[7] bring about a depression of the cardiovascular system.

Recently it has been reported that the area postrema, which lies outside the blood-brain barrier[8] and protrudes into the fourth ventricle, mediates the central cardiovascular response to angiotensin II in the dog[9]. The area postrema bears a close histological resemblance to the carotid body[10] and has been shown to act as a chemoreceptor[11]. We have destroyed the area postrema of the rat and report here the effect of this operation on the blood pressure.

Male Sprague-Dawley normotensive rats and spontaneously hypertensive (SH) rats of the strain developed by Okamoto and Aoki[12] were used. The destruction of the area postrema was performed by sight through the occipital foramen by thermocoagulation. The procedure has been described in detail previously[13]. With the exception of thermocoagulation, the sham operation was exactly similar to the true operation. The blood pressure in unanaesthetised rats was measured indirectly by a tail cuff method[13] and in anaesthetised animals (pentobarbital, 40 mg kg^{-1}, i.p.) directly from the carotid artery.

In 1 week the ablation of the area postrema induced an elevation of blood pressure both in initially normotensive (Fig. 1) and in SH rats (Fig. 2). Five weeks after the operation the blood pressure of animals with the area postrema destroyed was still greater than that of the controls. Although the rats were accustomed to the blood-pressure measurements before the operation, most of them were somewhat excited at the start of each measurement, that is for the first 2 to 3 min. At 1 week the first recordings in normotensive controls were 7±2 mm Hg ($n=15$) higher than the stabilised values 3 to 4 min later (Fig. 1), whereas in the area postrema ablated rats, the initial values were 17±3 mm Hg ($n=18$) higher than the stabilised ones. In SH rats, the corresponding differences were 9±4 mm Hg ($n=14$) and 30±5 mm Hg ($n=19$), (Fig. 2). In sham-operated rats the greatest difference between the first recordings and the stabilised value was 15 mm Hg, but in rats with the area postrema destroyed differences up to 60 mm Hg were measured. The initial pressure recordings remained higher than the later ones for at least 2 weeks. During the first measurement procedures, some of the operated rats died of pulmonary oedema. Shortly before death in SH rats, values up to 270 mm Hg, and in initially normotensive rats up to 200 mm Hg, were measured. No such blood pressure elevations or sudden deaths occurred in the sham-operated rats.

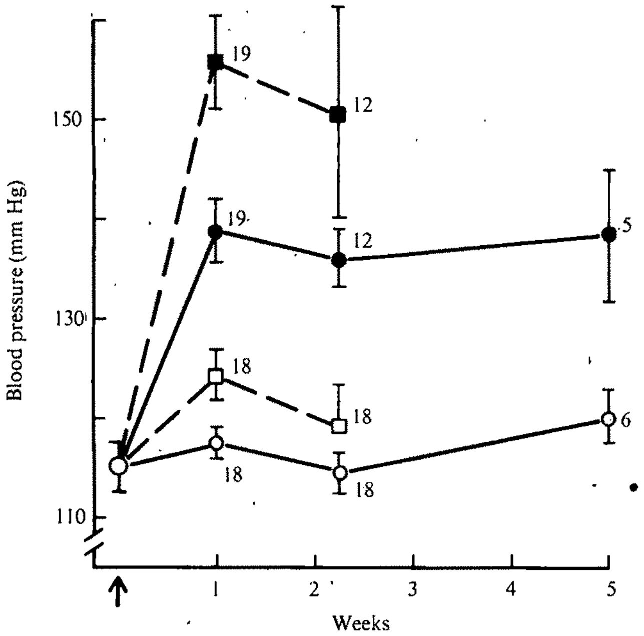

Fig. 1 Influence of the ablation of area postrema (■,●) and sham operation (□,○) on the blood pressure of normotensive rats. – – –, Values recorded during the initial 2 to 3 min period of the measurement procedure; ——, the following, stabilised values. Vertical bar, s.e.m. The number of rats is given on the figure.

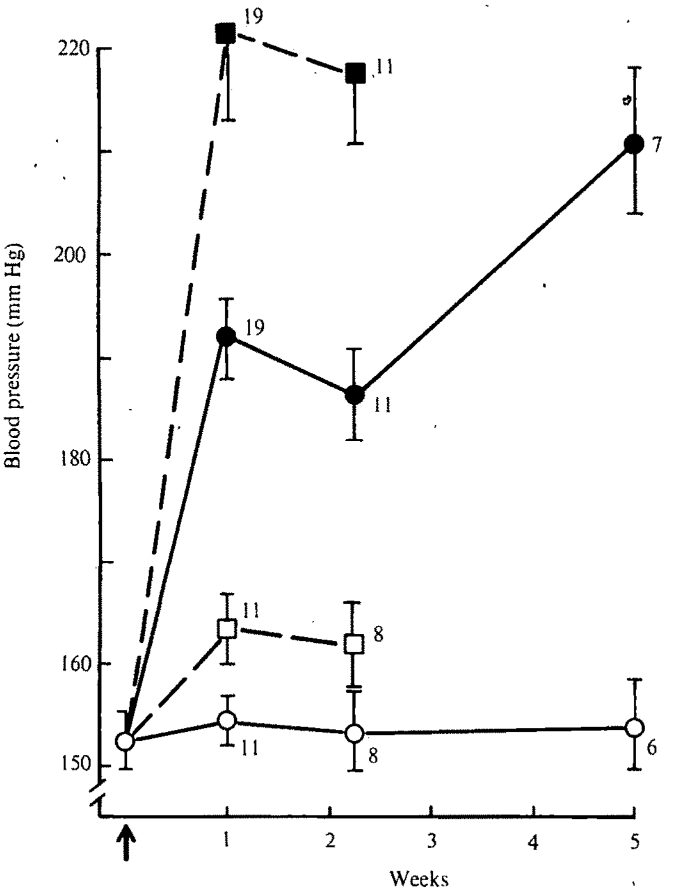

Fig. 2 Influence of the ablation of area postrema (■,●) and sham operation (□,○) on the blood pressure of spontaneously hypertensive rats. Symbols as in Fig. 1.

About 3 weeks after the destruction of the area postrema the initially normotensive rats also showed an elevated blood pressure during anaesthesia (sham operated, 126±8 mm Hg ($n=9$); area postrema ablated, 147±6 mm Hg ($n=11$), $P<0.05$).

The area postrema may exert a tonic inhibition on the blood pressure in the rat as its ablation induced an elevation of the blood pressure lasting at least 5 weeks. The area postrema might also be involved in the feed-back control of blood pressure as, in the absence of this region, very high blood pressures were induced by a stress situation, that is, by the recording of blood pressure.

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Mechanism of the Viscoelastic Deformation of Collagenous Tissue

THE purpose of the experiment reported here was to examine the striking observation of Rigby *et al.*[1] that for collagenous tissue the thermally activated process controlling mechanical relaxation changes abruptly at the body temperature. For rat tail tendon it was observed[1] that below 37° C the process is entropy activated: the activation energy $\Delta H=0$. Above 37° C, Rigby *et al.*[1] conclude that ΔH is large although their

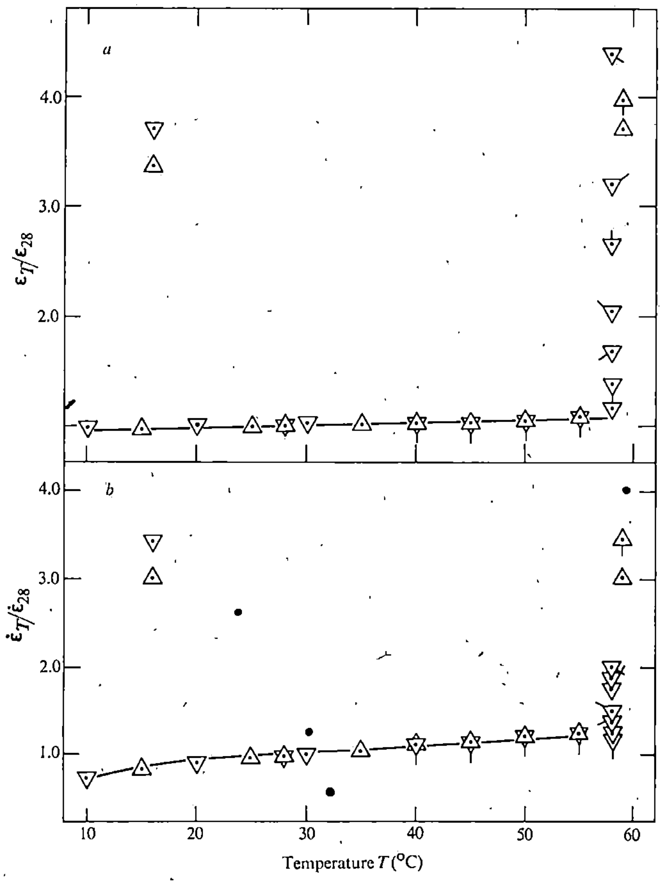

Fig. 1 Temperature dependence of normalised isochronal strain (*a*) and isochronal strain rate (*b*) at $t=20$ s for two specimens of digital tendon. Repeated experiments at a given temperature are indicated by the pips on the triangles, beginning with pip downwards for the second experiment and progressing clockwise by 60° for each successive experiment. Tendons studied under constant loads of 0.72 kg (male, 55 yr) and 0.30 kg (female, 89 yr). *a*: ▽, Male, $\varepsilon_{28}=1.40\%$; △, female, $\varepsilon_{28}=1.42\%$. *b*: ▽, Male, $\dot{\varepsilon}_{28}=5.81\times10^{-3}\%\ \text{s}^{-1}$; △, female, $\dot{\varepsilon}_{28}=4.00\times10^{-3}\ \%\ \text{s}^{-1}$.

experiment does not yield a quantitative result. The result is paradoxical in two ways. First, entropy activation, although feasible conceptually, is essentially unknown in nature. Second, is it plausible that the rate process should change in such a profound way right at the body temperature? We have now resolved both paradoxes.

The measurements were made on human flexor digitorum sublimus tendon *in vitro*. The specimen was mounted in a creep apparatus in which it could be subjected to a constant tensile load, and the tensile strain measured as a function of time of application of load, *t*. The specimen was immersed in 0.9% saline solution buffered to *p*H 7 which was maintained at a constant temperature in the range 10° C to 60° C. Figure 1 shows data for two specimens, one from a male of age 55 yr and the other a female of age 89 yr. The strain at $t=20$ s observed at *T*, ε_T, is divided by the strain at $t=20$ s observed at 28° C, ε_{28}, and plotted against *T* in Fig. 1*a*. It will be seen that (1) there is no discontinuity in the region of 37° C; (2) the data for both specimens are in excellent agreement; and (3) there is a transition at 58° C. The temperature dependence of the creep rate $\dot{\varepsilon}_T$ supports these conclusions. This is illustrated in Fig. 1*b* in which $\dot{\varepsilon}_T/\dot{\varepsilon}_{28}$ is plotted against *T*.

It may be concluded that for digital tendon there is no clear evidence for a change in rate process at 37° C; however, in order to test further the conclusion of Rigby *et al.*[1] that $\Delta H=0$ below 37° C we measured ΔH at 28° C by observing the change in creep rate induced by a small abrupt change of temperature (*T*-jump experiment). It follows from the theory of reaction rates[2,3] that if during a creep experiment the temperature is changed abruptly from T_0 to T at time t' and if $\dot{\varepsilon}_{T_0}$ and $\dot{\varepsilon}_T$ are the measured strain rates at T_0 and T at time t', then

$$\dot{\varepsilon}_T/\dot{\varepsilon}_{T_0}=\exp[(\Delta H/R)\,(1/T_0-1/T)] \tag{1}$$

Hence,

$$\ln\,(\dot{\varepsilon}_T/\dot{\varepsilon}_{T_0}) = (\Delta T/RT\,T_0)\,\Delta H \tag{2}$$

in which $\Delta T=(T-T_0)$ and R is the gas constant. In our experiment a number of *T*-jumps were performed from a fixed $T_0=28°$ C. According to equation (2) a plot of $\ln(\dot{\varepsilon}_T/\dot{\varepsilon}_{T_0})$ against $\Delta T/RT\,T_0$ should be linear and of slope ΔH. In each experiment the temperature was changed at a time $t'=60$ s after application of the stress. Fig. 2 shows results for the two specimens already described and a specimen taken from a male of 21 yr. It will be seen that the data for all three specimens are in good agreement and yield a finite value of ΔH, 12 kcalorie mol^{-1} at $T_0=28°$ C.

It follows from this result that for human digital tendon the conclusion of Rigby *et al.*[1] does not hold. The discrepancy between the two experiments is difficult to explain. It is certainly not due to the use of different mechanical techniques, stress relaxation[1] as opposed to creep. It is quite possible that since ΔH is low, and since Rigby *et al.*[1] studied many specimens (rat tail tendon breaks easily in saline solution and for this reason was not used in our experiments), the random scatter of results obscured the small temperature dependence of the stress relaxation modulus. This would explain the conclusions of Rigby *et al.* below 37° C. The cause of the sudden increase in the temperature dependence of stress relaxation modulus above 37° C observed by Rigby *et al.*[1] in rat tail tendon is unexplained. It could be due to the age of the tendon, since their specimens were taken from animals of age approximately 3 months.

A complete description of our work will be presented elsewhere including a study of the shrinkage transition at 58° C using the *T*-jump technique. This transition is rate activated with $\Delta H\sim250$ kcalorie mol^{-1}. It is our belief that for pseudo-first order transitions of this type and also for mechanical relaxation the *T*-jump technique is of considerable potential

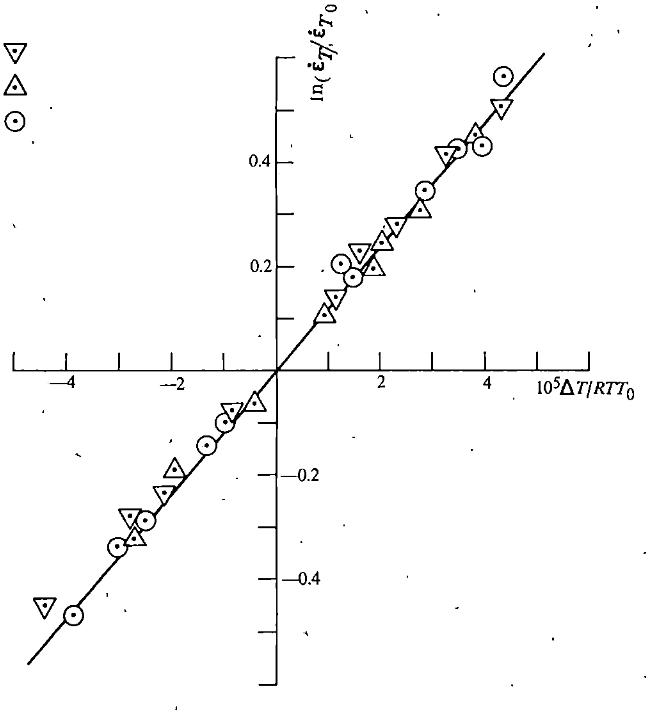

Fig. 2 Dependence of ln $\dot{\varepsilon}_T/\dot{\varepsilon}_{T_0}$ for three specimens of digital tendon on $\Delta T/RT\,T_0$. Straight line drawn according to equation (2) with $\Delta H=12$ kcalorie mol^{-1}. Tendons studied under constant loads of 0.72 kg (male, 55 yr (▽)), 0.30 kg (female, 89 yr (△)) and 0.76 kg (male, 21 yr (⊙)). $T_0=28°$ C.

for the study of biological systems. The linear viscoelastic techniques such as time–temperature superposition, which have found wide application for determining ΔH in synthetic polymers[4,5], are not applicable to biological systems. The strength of the *T*-jump technique is that even when the structure of the specimen changes during the deformation process (as it does in tendon[6]) the activation energy may still be determined since the creep rates $\dot{\varepsilon}_{T_0}$ and $\dot{\varepsilon}_T$ are determined at the same structure: that which occurs at the time of the *T*-jump. The technique is also accurate for low values of ΔH when superposition (as in the experiments of Rigby *et al.*[1]) becomes inaccurate.

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Ion Beam Etching of Red Blood Cells and Latex Spheres

ION etching techniques have recently been utilised in attempts to reveal subsurface cell structures in the scanning electron microscope[1–7]. There has, however, been some difficulty in distinguishing intracellular structures from artefacts produced by the ion etching process. This inability to distinguish cellular structures from features produced by the ion beam suggests that a better understanding of the ion etching process as it is applied to organic material is required before this technique can be applied to the recognition and investigation of biological cell types and structure. In our study we examined the etch effects produced in normal red blood cells and homogeneous latex spheres by a variety of ion beam etching conditions.

Initial glow discharge ion etching studies of red blood cells showed that the etched red cell consisted of cone-like or filament-like structures[1]. Later studies[2,5–7] demonstrated that these filament-like structures and similar ridge-like features found in other etched cells[2–6] and tissues[7] were produced by the ion etching process[2]. The presence of these cone-like structures in ion etched cells and tissue has hindered the identification of cellular components. The study reported here was carried out to determine the ion etching conditions which reduce cone formation in red blood cells and homogeneous latex spheres.

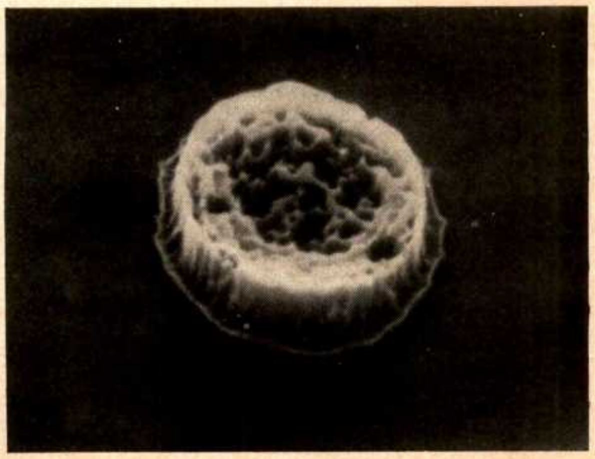

Fig. 1 Normal red blood cell ion beam etched by a 6 kV argon ion beam at 90° angle of incidence for about 25 min.

The red cell fraction of normal whole blood was washed in an isotonic phosphate-buffered medium (10 mM KCl, 141 mM NaCl, 1 mM $MgCl_2$, 1.3 mM $CaCl_2$, 0.8 mM NaH_2PO_4, 5 mM Na_2HPO_4 at *p*H 7.4, 300 mOsm) and fixed for 30 to 60 min in 0.5% phosphate-buffered glutaraldehyde, *p*H 7.4, 320 mOsm. The cells were then washed in deionised water and allowed to settle from a droplet of water onto a silicon disk. Care was taken that air drying did not occur during this time or during subsequent procedures. Dehydration was carried out by bathing the cells in 20, 50, 70, 90 and 100% ethanol solutions and 50 and 100% amyl acetate solutions for 5 min periods. The cells were taken directly from the 100% amyl acetate solution and critical point dried in carbon dioxide.

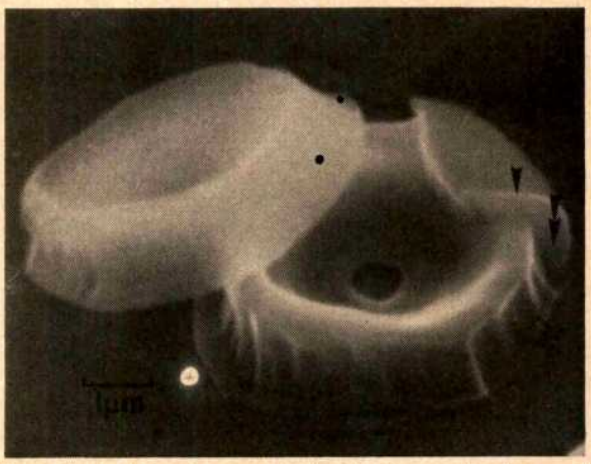

Fig. 2 Red cell ion beam etched by 17 kV argon ion beam with 90° angle of incidence for 3 min. A portion of the bottom cell which was shielded from the ion beam is unetched.

Styrene divinylbenzene copolymer latex spheres, 4 to 8 µm in diameter, were obtained from the Dow Chemical Company. The latex spheres were dispersed in deionised water, deposited on a silicon disk and air dried.

Ion beam etching was carried out in the specimen chamber of an ETEC Autoscan scanning electron microscope using an ETEC ion beam etching device with argon gas. The ion beam had an estimated diameter of 2 mm and a 2.3° beam divergence. The specimen was positioned about 1 inch from the ion gun aperture. Ion accelerating voltages of 5 to 20 kV with ion currents of 0.5 to 20 µA, respectively, were used for etching, The angle of incidence was maintained at 90° and etching times ranged from 1 to 30 min.

After etching, the red cells and latex spheres were coated with thin films of carbon and gold or gold-palladium and examined in the scanning electron microscope at 20 kV.

Red blood cells etched by a 6 kV argon ion beam had a very irregular surface characterised by cone- or ridge-like structures (Fig. 1) similar to those seen in cells etched by 1 to 4 kV argon ion beams[2,6]. There has been some indication in previous studies that the texture of the etched cell surface becomes smoother as the ion beam accelerating voltage increases[2]. The change in the texture of the etched cell surface with increasing ion accelerating voltage can be seen by comparing the cell in Fig. 1 with the cells in Fig. 2 which were etched by a 17 kV argon ion beam. Although the cells in Fig. 2 lacked much of the surface structure present in the cells in Fig. 1, the cells in both figures had similar groove-like features along their perimeter. These grooves were always found to be oriented in the direction of the ion beam.

The configuration of the cells in Fig. 2 during etching was such that a portion of the bottom cell was shielded from the ion beam. It is possible to see a cross-section of the portion of the cell that remained unetched. The shape of the etched sections of the cells suggests that different amounts of material were removed from different areas of the cells. It seems that less material has been removed from areas where the ion beam intersected the cell surface at 90° (indicated by single arrow) than from areas where the ion beam had about a 45° angle of incidence with the surface of the cell (double arrow).

The variation in the sputtering yield (number of atoms removed for each incident ion) with angle of incidence suggested in Fig. 2 is similar to the results of earlier studies of ion beam etching of metals[8]. The shapes of etched metal spheres inserted into ion beams demonstrated that the sputtering yield was a minimum at 90°, increased to a maximum at from 20° to 40°, and decreased again at grazing incidence.

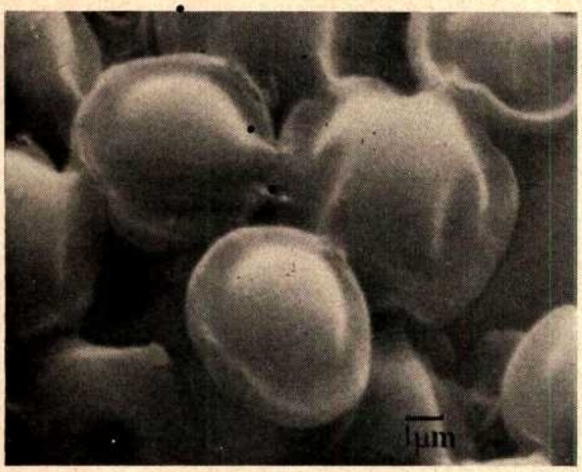

Fig. 3 Latex spheres etched by a 17 kV argon ion beam at 90° angle of incidence.

The effects of the change in sputtering produced by a change in angle of incidence along a curvilinear surface can also be seen in etched latex spheres (Fig. 3). Ion beam etched latex spheres are comparable to metal spheres which were ion beam etched in earlier studies[8]. The surfaces of the etched latex spheres had a smooth texture similar to that of red cells etched under similar conditions (Fig. 2) and occasionally had groove-like features somewhat similar to the features along the perimeter of the etched red cells.

The results of this study demonstrate that cone- and ridge-like structures present in red cells etched by relatively low voltage (1 kV to 6 kV) ion beams are reduced as the ion accelerating voltage is increased (for example, to 17 kV). The results further demonstrate that the shapes of red blood cells and homogeneous latex spheres etched with ion beams are comparable and can be explained by the change in the sputtering yield with the variation of the angle of incidence along the red cell and latex sphere surface. This relationship between sputtering yield and angle of incidence is similar to that determined earlier for inorganic materials. The results of the red cell etching are consistent with the view that the normal red cell is homogeneous, having no distinct subsurface structure resolvable in the scanning electron microscope. Work now in progress is using the ion beam etching technique to study the structure of nucleated and parasitised red cells.

This work was completed in part while M. S. was a US National Aeronautics and Space Administration-American Society for Engineering Education Summer Faculty Fellow in the Cellular Analytical Laboratory of the Lyndon B. Johnson Space Center, Houston, Texas.

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Optically Detected Conformational Changes in Haemoglobin Single Crystals

THERE is considerable X-ray evidence that the haem stereochemistry is different in oxy- and deoxyhaemoglobin[1-3]. This structural change is an important part of the oxygenation process, because it is believed to induce conformational changes in the surrounding protein that are responsible for cooperativity. We have investigated conformational changes in the haem region by measuring polarised absorption spectra on single crystals. Using this technique we have detected changes in porphyrin plane orientation associated with an alteration of the ligand, spin, and oxidation state of the haem iron, factors which influence haem stereochemistry. Our results not only show for the first time that contacts between the porphyrin and amino acid side chains change upon oxygenation, but they are also consistent with the Hoard-Perutz hypothesis on the nature of the stereochemical trigger[1-3].

Crystals of horse oxyhaemoglobin, carbonmonoxyhaemoglobin, and methaemoglobin were prepared according to Perutz[4], except that the oxy and carbonmonoxy forms were crystallised at 4° C in order to minimise oxidation to methaemoglobin. Crystals of cyanide, azide, fluoride, and formate complexes were formed by diffusing the ligand into the methaemoglobin crystal. The deoxy form was prepared by dithionite reduction of methaemoglobin crystals previously modified with bis(N-maleimidomethyl)ether (BME)[5]. Single crystal spectra were measured over a wide wavelength range (230–1,200 nm) with plane-polarised light incident normal to the (001) face of the horse haemoglobin crystal[4] of space group C2. The measurements were made with a microspectrophotometer similar to the one previously described[6].

The important experimental parameter of the single crystal spectrum is the polarisation ratio. It is defined at each wavelength as the ratio of the optical density measured with the electric vector of the plane-polarised light aligned parallel to the *b* crystal axis to the optical density measured with the electric vector parallel to the *a* crystal axis. The polarisation ratio in the purely haem absorption region depends only on geometric factors. These are the molecular directions of the haem complex in which plane-polarised light is maximally absorbed and the orientation of these directions with respect to the *a* and *b* crystal axes. For the intense Soret band near 400 nm these molecular directions are very accurately described in all derivatives by a plane parallel to the mean plane of the 24 porphyrin ring atoms. This means that no light is absorbed for the component of the electric vector perpendicular to the porphyrin plane, while all directions contained in this plane show equal absorption. This planar-absorber behaviour for the Soret band has been established from the excellent agreement of the optically-determined porphyrin plane orientation[7–9] (Makinen and Eaton, unpublished) with that found by X-ray diffraction[10–14] and electron paramagnetic resonance[15] studies on a variety of haem protein crystals. Thus, we interpret differences in Soret polarisation ratio among haemoglobin derivatives as arising from differences in porphyrin plane orientation.

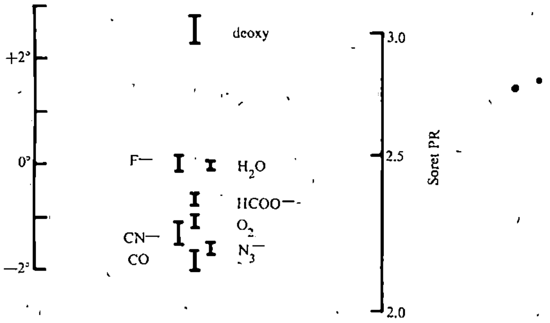

Fig. 1 Soret polarisation ratio and relative porphyrin plane orientation in horse haemoglobin derivatives. The uncertainties represent the average deviation from the mean of measurements on at least two crystals. The minimum angle between porphyrin plane normals, φ, is calculated from equation (1) and is relative to the porphyrin normal of (aquo) methaemoglobin. The following abbreviations are used: deoxy (deoxyBMEhaemoglobin), H_2O (aquomethaemoglobin), O_2 (oxyhaemoglobin), CO (carbonmonoxyhaemoglobin), CN^- (cyanomethaemoglobin), F^- (fluoridomethaemoglobin), $HCOO^-$ (formatomethaemoglobin), N_3 (azidomethaemoglobin).

Our results are summarised in Fig. 1. The scale at the right is the average polarisation ratio for the Soret band. The scale at the left shows the minimum change in orientation of the porphyrin plane that can account for the observed change in polarisation ratio. The angles are referred to the porphyrin normal of methaemoglobin and are calculated from the relation

$$\varphi_{ij}=\arctan (PR)_i^{\frac{1}{2}}-\arctan (PR)_j^{\frac{1}{2}} \qquad (1)$$

where $(PR)_i$ and $(PR)_j$ are the Soret polarisation ratios for the ith and jth derivatives. In deriving this expression we have assumed D_2 (222) symmetry for the orientation of the four porphyrin planes in all derivatives. This symmetry is exact for methaemoglobin within the experimental errors of both the electron paramagnetic resonance results[15] and the 2.8 Å atomic coordinates provided to us by M. F. Perutz. It is important to note that any deviation from D_2 symmetry for either the ith or jth derivatives will result in an increased absolute value of φ_{ij} for either α or β haems, or both. If we assume, for example, that only α or only β haems change orientation, then the absolute values of φ_{ij} are doubled. Also, changes in porphyrin plane orientation can be assumed to take place relative to axes fixed within the subunits since X-ray data[4,16,18] show that the quaternary structures of methaemoglobin, azidomethaemoglobin, oxyhaemoglobin, carbonmonoxyhaemoglobin, and deoxyBMEhaemoglobin are essentially identical.

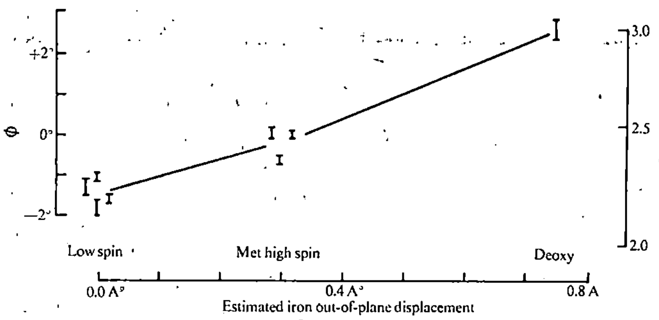

Fig. 2 Soret polarisation ratio and relative porphyrin plane orientation against estimated displacement of the iron from the mean porphyrin plane. The displacement in horse methaemoglobin and deoxyhaemoglobin is 0.3 Å and 0.75 Å, respectively[2]. To assign approximate values for the other derivatives we have appealed to the X-ray data on sea lamprey cyanomethaemoglobin[19] and the predictions from small molecule structures[3]. Thus, we use 0.75 Å, 0.3 Å, and 0.0 Å for ferrous high spin (deoxy), ferric high spin (H_2O, F^-, $HCOO^-$), and ferric or ferrous low spin derivatives (O_2, CO, CN^-, N_3), respectively. The polarisation ratios (PR) are shown as clustered around these points for clarity.

Our interpretation of the variation in Soret polarisation ratio among haemoglobin derivatives receives strong support from X-ray results. The minimum angle between the porphyrin normals of methaemoglobin and deoxyhaemoglobin is calculated to be +2.5° (Fig. 1). This value is consistent in both sign and magnitude with the movement of the porphyrin planes detected by difference Fourier studies[2,16] (M. F. Perutz, personal communication). The only other haemoglobin derivative to be examined in the liganded quaternary structure at high resolution by X-ray methods is horse carbonmonoxyhaemoglobin. Here again the optical and X-ray results are in agreement (E. G. Heidner, personal communication). Oxyhaemoglobin has not yet been studied at high resolution because the crystals are almost completely oxidised to methaemoglobin by the end of the X-ray exposure[2]. The much smaller crystals used in our optical experiments contained less than 20% methaemoglobin. We find that the porphyrin planes tilt at least 3.5° in the transition from oxyhaemoglobin to deoxyhaemoglobin constrained by BME to remain in the liganded quaternary structure. This rotation corresponds to a displacement of peripheral porphyrin atoms of at least 0.3 Å. Since there are a large number of non-bonded interactions between the protein and porphyrin—there are approximately sixty atoms of the protein less than 4.0 Å from the porphyrin[20]—such a displacement must be accompanied by a movement of amino acid side chains in contact with the porphyrin ring. Thus, our observation of a change in orientation of the porphyrin plane supplies the first experimental evidence for a change in porphyrin-protein contacts upon oxygenation.

Hoard[1,3] and Perutz[2,16] have suggested that the important structural change initiating cooperativity in the protein is a shortening of the distance between the haem-linked histidine and the porphyrin plane upon oxygen binding. The histidine is covalently bonded to the iron, so that the principal part of this movement arises from the shift of the iron from 0.75 Å out of the plane in deoxyhaemoglobin to an essentially coplanar position in oxyhaemoglobin. This histidine-porphyrin movement, referred to as the stereochemical trigger, is expected to force changes in the contacts between the porphyrin and amino acid side chains[16]. From this stereochemical trigger hypothesis we would predict that derivatives with similar iron out-of-plane displacements should have similar porphyrin orientations. As a test of this prediction we have plotted our orientation results against the estimated iron out-of-plane displacement (Fig. 2). The data do indeed neatly divide into three classes according to the iron-porphyrin distance. This result is not only consistent with the postulated importance of the histidine-porphyrin movement, but it also suggests that this stereochemical change is transmitted to the protein through an alteration in porphyrin-protein contacts.

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Fluoridated Hydroxyapatite Solubility and Caries Formation

SINCE the early observations[1] on reduction of caries with increasing fluoride concentrations in drinking water, the tacit assumption was made that fluoride incorporated in dental enamel acts as a cariostatic agent. An appealing explanation for the possible mechanism involved is based on the nature of the enamel mineral of which hydroxyapatite (OHA), $Ca_5OH(PO_4)_3$, is considered to be the prototype. Replacement of fluoride for the hydroxyl would yield fluorapatite (FA) which has a lower solubility. Analyses of surface layers of enamel, however, show that the fluoride contents are far below the level expected for FA. It is relevant then to ascertain to what extent partial substitution of the fluoride for the hydroxyl group in HA affects its solubility.

In a recent publication[2] related to the anti-cariogenic effect of fluoride, Driessens reported calculated solubilities of the solid solutions $Ca_5(OH)_{1-x}F_x(PO_4)_3$ of which hydroxyapatite and fluorapatite are the end members. The subscript x is the degree of substitution of fluoride for hydroxyl and its numerical value varies from 0 (for HA) to 1 (for FA). His calculations are based on the standard free energies of formation ΔG_f° (free enthalpies in Driessens's terminology) reported by Duff[3]. These latter values are open to question because their calculation was based on analytical data for systems which were not demonstrated to be at equilibrium and because analytical data were used to calculate ΔG_f° values for compounds that were not formed in the experimental systems.

We have prepared a series of solid solutions with degrees of fluoride substitution, x, estimated chemically, was 0, 0.13, 0.24, 0.41, 0.57, 0.81, 0.89 and 1; details about the preparation of these compounds, involving a very slow precipitation process, will be published elsewhere. The solid-solution nature of these samples was verified by crystallographic techniques. Thus, a linear decrease in the magnitude of the unit cell parameter, a, was obtained with increasing values of x. As shown below, however, the thermodynamic properties of these compounds are slightly different from those expected for an ideal solid solution and, as should be anticipated[4], these small differences significantly affect their solubility behaviour.

We report here the solubility of these compounds obtained by equilibrating them for more than 1 month with a series of dilute phosphoric acid solutions at 37° C. For a given member of the series we can express its solubility product constant, K_x, as $K_x=(Ca^{2+})^5(OH)^{1-x}(F^-)^x(PO_4{}^{3-})^3$ in which parentheses indicate activities. The ionic activities in the liquid phase can be calculated by iterative procedures similar to those previously reported[8]. Introducing the definition of the ionisation constant of water, K_w, it can be shown that $\log (H+)^3 (PO_4{}^{3-})=1/3 \log K(x,w) -5/3[\log(Ca^{2+})(OH^-)^2+x/5 \log (H^+)(F^-)]$ in which $K(x,w)=K(x) K(x)^{(9+x)}$. Thus, a plot of the quantity $-\log (H^+)^3(PO_4{}^{3-})$ against the quantity $-x/5 \log (H^+)(F^-) -\log(Ca^{2+})(OH^-)^2$ should yield a straight line with a slope of $-5/3$. The plots for the various compounds will be displaced depending on the value of x but the slopes should be the same. The results of the analyses of the aqueous phase after equilibration were used to obtain the plots shown in Fig. 1. It is apparent that the data yielded a series of straight lines with the anticipated slope, within experimental error. These results constitute verification of the equilibria between the solid and liquid phases involved.

In Table 1 we report the values of K_x for the various degrees of substitution. A polynomial fit to these data (degree of determination 0.95) shows a minimum in K_x for a degree of substitution of 0.56, implying a minimum in the ΔG_f° for such a composition. The calculations reported by Duff[3] do not show a minimum. This minimum, experimentally determined, could be anticipated on the basis of the disordered structure[5,6] of hydroxyapatite and the hydrogen bonding[7] of the hydroxyl

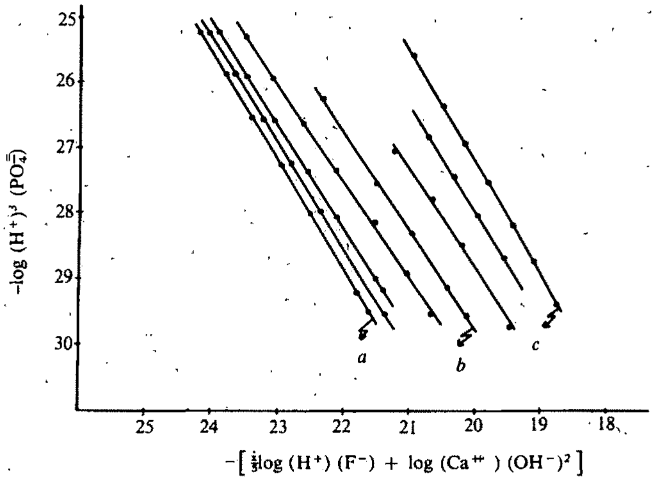

Fig. 1 Plots of quantities derived from solution compositions at equilibrium (see text). The slopes of the various lines (5/3 within experimental error) satisfy the equilibrium requirements. *a*, FA; *b*, $x=0.405$; *c*, OHA.

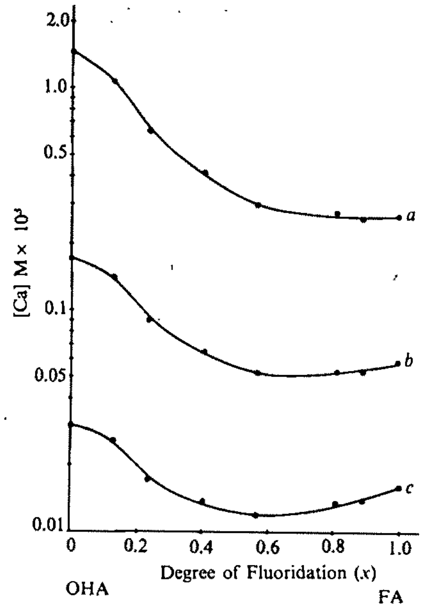

Fig. 2 Solubility of the solid solutions (expressed as molarity of calcium) as a function of the degree of fluoride substitution. Results are shown for, *a*, *p*H 5; *b*, *p*H 6; *c*, *p*H 7.

group to fluorine occurring in the structure. Both of these features would make their greatest contribution to the stability of the crystalline lattice at a degree of substitution of about 0.5.

Considering the constituent ions of the solid solutions plus the ion pairs[8] $CaH_2PO_4^+$ and $CaHPO_4^0$ as the only species present in the equilibrated solution, we have constructed the plots shown in Fig. 2. On the ordinate are plotted the calcium concentrations in the aqueous phase maintained by the solid solution at equilibrium. Therefore, the curves reflect the variation of the solubility as a function of the degree of substitution. Values for x in the order of 0.05 are usually obtained for the surface enamel from fluoridated areas by assuming that the fluoride is uniformly distributed in the enamel sample. Contrary to the assertion by Driessens[2], the plots show that substitutions of this order (0.05) have very little effect on the solubility of hydroxyapatite. The fluoride concentration gradient is very steep[9,10], however, in the first few micrometres of enamel. Incidentally, this gradient is already established before tooth eruption; the fluoride concentrations in enamel from fluoridated areas are of a pre-eruptive origin[10] and not as a result of topical effects as Driessens has suggested[2]. In fact, recent work[11] on analyses of very shallow biopsis (0.2–0.4 µm) indicates that it is not unreasonable to consider that the degree of substitution at the very surface of the enamel may approach a value close to 0.5. If this is the case, our results indicate a significant reduction in solubility which undoubtedly could contribute to the cariostatic effect of fluoride. Important as this reduction in solubility may be in relation to caries prevention, there are other aspects that should be considered. For example, fluoride intake at early ages (while teeth are in the formative period) produces shallower pits and fissures in occlusal surfaces[12]. Furthermore, it appears that the electrochemical properties of the surfaces of fluoridated apatites are different from those of hydroxyapatite. Thus, it has been reported[13] that the adsorption of salivary proteins is significantly different between these two kinds of compounds. We have verified this behaviour in our own laboratories. It is conceivable therefore that the selective formation of permselective coatings induced by the fluoridated surfaces of enamel may enhance the protection of the tooth structure.

Table 1 Values of K_x for Various Degrees of Substitution

Degree of substitution (x)	Solubility product constant (K_x)
0.0	$7.36 \pm 0.93 \times 10^{-60}$
0.13 ± 0.004	$2.13 \pm 0.11 \times 10^{-60}$
0.24 ± 0.01	$8.44 \pm 2.59 \times 10^{-62}$
0.41 ± 0.01	$2.32 \pm 0.81 \times 10^{-62}$
0.57 ± 0.02	$6.55 \pm 2.17 \times 10^{-63}$
0.81 ± 0.03	$5.87 \pm 1.30 \times 10^{-62}$
0.89 ± 0.03	$7.99 \pm 0.90 \times 10^{-62}$
1.00 ± 0.03	$3.19 \pm 0.14 \times 10^{-61}$

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Reversal by Narcotic Antagonist of a Narcotic Action elicited by a Conditional Stimulus

THERE is considerable evidence to indicate that learning is important in narcotic dependence[1]. However, there has been little animal research into the mechanism of the interaction between environmental stimuli and drug action which maintains dependence. In morphine-dependent rats[2] and monkeys[3] conditional stimuli previously associated with withdrawal can elicit physiological disturbances characteristic of narcotic withdrawal. Also, during withdrawal the conditional stimuli previously associated with morphine substitution can elicit blockade of morphine withdrawal[4]. In an experiment by Roffman *et al.*[4], after a number of pairings with morphine injections, a neutral stimulus (bell) elicited a blockade of withdrawal hypothermia, a task normally accomplished by morphine itself. It was then suggested that there is an overlap between the brain mechanisms sensitive to morphine action and those affected by conditional stimuli[5]. Therefore, repeated pairing of hyperthermic effects of morphine with the stimulus-induced neural activity results in the conditioning of the former to the latter. We reasoned that if the brain activity due to a conditional stimulus evokes activity of the morphine receptors, a morphine antagonist should reverse the effectiveness of that stimulus in mimicking the morphine action. To obtain evidence in favour of this hypothesis we used naloxone, a specific antagonist of morphine action[6].

We used male Long-Evans rats (200–250 g) housed individually in a room maintained at 21–23° C with lights alternating on a 12 h dark–light cycle. Food (Purina chow) and water were available *ad libitum* except during injections and when physiological measurements were being made. The rats were given two equally spaced injections of morphine sulphate daily in increasing doses until a maintenance dose was reached. The first injection consisted of 10 mg kg^{-1}, which was increased by 10 mg kg^{-1} every day, until the tenth day, when each injection consisted of 100 mg kg^{-1}. The rats were maintained at this dosage (200 mg kg^{-1} d^{-1}) for 3–5 d and then withdrawn. Each morphine injection was given in a well-lit room which was sound-attenuated and maintained at a constant temperature of 21° C. Each injection was paired with a bell which was turned on 1 min before the injection and turned off at the completion of that injection. After each injection, the rat was returned to its home cage away from the injection room.

During the withdrawal period of 24 h these rats were divided into three groups. One group (thirty-six rats) was not given any treatment during abstinence to obtain withdrawal hypothermia. A second group (six rats) was injected with morphine and exposed to the bell as during the addiction phase to serve as non-withdrawal controls. The third group (six rats) also continued to be injected and exposed to the bell as during the addiction phase. In this case, however, saline was injected instead of morphine sulphate to obtain the effect of conditioned stimuli during withdrawal from morphine. Thirty minutes after the last treatment, or after 24 h without treatment in the case of group 1, rectal temperature was measured by inserting a thermocouple probe 5 cm inside the rectum for 60 s. Following that measurement, the first group was divided into six subgroups (six rats each). One subgroup was then injected with naloxone (2 mg kg^{-1}), another with morphine (100 mg kg^{-1}), a third was exposed to the bell, and a fourth to the bell followed by naloxone given 10 min after the bell. The fifth group was given morphine followed by naloxone 30 min later. The last group was not given any treatment to serve as a control for withdrawal hypothermia.

It was previously established[4,5] that the bell paired with saline does not affect rectal temperature in non-addicted rats. Also, naloxone did not cause any change in temperature of the non-addicted rats.

Table 1 Effect of Conditional Bell and Morphine on Rectal Temperature of Morphine-dependent Rats during Abstinence

Treatment during 24 h abstinence*	*N*	Rectal temperature (°C)† mean ±s.e.
None	36	37.42±0.03
Bell and morphine‡	6	39.06±0.06
Bell and saline	6	38.06±0.06

The rats were addicted by repeated injections of morphine paired with bell.

* After a maintenance dose of 100 mg kg^{-1} (total daily dose of 200 mg kg^{-1}) the morphine injections were withheld for 24 h and one of the three treatments applied.

† Measured 30 min after the indicated treatment.

‡ Same dose as during maintenance (100 mg kg^{-1}).

Morphine injection reversed the withdrawal hypothermia and produced hyperthermia in these rats (Table 1) as was previously reported by others[7]. Exposure to the bell without morphine also reversed the withdrawal hypothermia as expected from our previous study[4,5]. In a pilot experiment, presentation of the bell during abstinence or after morphine injection had not affected rectal temperature of a group of animals which had been repeatedly exposed to a bell during addiction phase, which was not regularly paired with morphine injections.

Data summarised in Table 2 show the effect of naloxone on the rectal temperature of the addicted rats undergoing withdrawal. In these rats naloxone without any pretreatment produced slight hypothermia. However, when these rats were injected with naloxone after the bell, which itself increased rectal temperature by a degree, naloxone caused a severe decrease of that temperature. This effect of naloxone was similar to that seen after morphine injection in the addicted rats. Morphine caused marked hyperthermia in the abstinent rats. Naloxone injected after morphine caused a considerable decrease of temperature in these rats.

Table 2 Effect of Naloxone, Morphine or Conditional Stimulus Administered after an Abstinence Period of 24 h in Morphine-addicted Rats

Test treatment*	*N*	Rectal temperature§ mean (°C)±s.e.	Change‖ (° C)	*P*¶
None	6	37.00±0.08	—	—
Naloxone†	6	36.68±0.06	−0.32	<0.05
Bell	6	38.04±0.06	+1.04	<0.001
Bell+ naloxone	6	36.17±0.06	−1.87	<0.001
Morphine‡	6	38.87±0.21	+1.87	<0.001
Morphine+ naloxone	6	37.37±0.26	−1.28	<0.001

* Given 24 h after last morphine injection.

† 2 mg kg^{-1}, given interperitoneally.

‡ Same dose as during the maintenance regime (100 mg kg^{-1}).

§ Measured 30 min after last treatment.

‖ From their respective control given in third column. Naloxone, morphine or bell group is compared with none group, while bell+naloxone group is compared with bell-alone group and morphine+naloxone is compared with morphine-alone group.

¶Student's *t* test, two tailed.

Either injection of morphine or exposure to the bell caused hyperthermia in the abstinent rats. The rectal temperature of morphine-treated rats was, however, higher than that of rats exposed to the bell. A quantitative similarity between morphine and the bell is not justified because we used a large dose of morphine (100 mg kg^{-1}) and attempted no variation of bell intensity for comparison.

Our findings on the ability of conditional stimulus to mimic the counter-withdrawal effect of morphine are in accord with earlier findings on conditioning of the agonistic effects of morphine. According to Bykov[8], Devlov and Petrova in Pavlov's laboratory used physical stimuli to elicit conditional electrocardiographic changes associated with morphine injection. Collins and Tatum[9] serendipitiously discovered a conditional salivary reflex associated with morphine injection. Kleitman and Crisler[10] replicated conditioning of salivary-reflex using morphine as the unconditional stimulus. It should be pointed out that our observations are limited to abstinence hypothermia and therefore our conclusions may not be applicable to other signs of abstinence. In rats, hypothermia has been considered one of the most reliable signs of abstinence.

Narcotic antagonists are known to precipitate narcotic withdrawal. As far as we know, however, interaction of narcotic antagonist with the learned conditional effects of morphine has not been previously studied. The ability of naloxone to block the physiological responses evoked by conditional stimuli in the same manner as it blocks the unconditional morphine effects has both theoretical and practical implications.

Of theoretical importance is the suggestion from this study that the conditional stimulus may evoke activity in the brain pathways specifically sensitive to the agonistic actions of morphine and to morphine dependence. Alternatively, the common belief that naloxone acts only by displacing morphine from its receptor may be questioned. Rather, naloxone may exert an agonistic influence on brain substrates originally insensitive to naloxone but rendered sensitive by the actions of morphine. Other experiments showing the inability of narcotic agonists to reverse actions of narcotic antagonists[11–13] raise similar doubts about the hypothesised mode of action of narcotic antagonists. A recent report on the naloxone antagonism of analgesia produced by brain stimulation[14] supports this possibility of the direct action of naloxone.

The practical importance of this finding is related to the use of narcotic antagonists in the therapy of narcotic addiction. The current rationale behind the use of narcotic antagonists in the treatment of heroin addicts is that treatment with these drugs will result in the extinction of heroin consumption because of the blockade of the 'high' sought from agonistic effects of illicit heroin. Our data suggest that narcotic antagonists may also be valuable in extinguishing heroin habit associated with the conditional placebo effects of heroin-seeking behaviour. These effects have been considered to be major factors in relapse to the addiction in humans[1].

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Survival of Common Bacteria in Liquid Culture under Carbon Dioxide at High Temperatures

MOLTON *et al.*[1] have reported that bacteria in liquid cultures survived exposure to normally lethal temperatures when heated in a high pressure CO_2 atmosphere. Because these results could influence planetary quarantine constraints, particularly for the planet Venus, and because no protection against thermal death by CO_2 has been reported previously, an attempt was made to verify their results.

In addition to the same strains of *Escherichia coli*, *Aerobacter aerogenes*, *Serratia marcescens*, and *Bacillus subtilis* used by Molton *et al.*[1], a thermophilic bacillus (YTG-2 S^r) and two genetically marked *E. coli* strains were tested. Initial experiments followed the methods for bacterial cultivation, CO_2 pressurisation, heating, and depressurisation described by Molton *et al.*[1], with the exception that YTG-2 S^r was grown at 65° C. Viability assays differed only in that dilutions beyond 10^{-6} were not used and duplicate rather than triplicate platings were made.

Stationary and log-phase cultures of *E. coli* B, *E. coli* K 12 S^r, *A. aerogenes*, YTG-2 S^r, *S. marcescens*, and *B. subtilis* were exposed to 160° C for periods of 3.5 to 22 h under 19.7 atm CO_2. No survivors were obtained. A less severe exposure to heat was also examined using stationary and log-phase cultures of *E. coli* B, *E. coli* B T-Pr-, and *B. subtilis*. No survivors were obtained with any of the three strains after exposure to 125° C for either 5 or 18 h under 9.5 atm CO_2 pressure.

Table 1 Effect of High Pressure CO_2 on the Survival of *E. coli* and *B. subtilis*

Organism	Pressure (atm)	Temperature ° C	Time (h)	Initial (Viable counts ml^{-1})	Final (Viable counts ml^{-1})	Survival (%)
E. coli B	9.5	22	1	2.7×10^9	3.4×10^9	125.9
E. coli B	19.7	22	1	2.7×10^9	3.3×10^9	122.2
E. coli B	19.7	15	18	2.7×10^9	3.1×10^9	114.8
B. subtilis	9.5	22	1	1.1×10^8	1.2×10^7	10.9
B. subtilis	9.5	22	1	1.4×10^8	4.7×10^7	33.6
B. subtilis	19.7	22	1	1.1×10^8	3.5×10^3	0.0032
B. subtilis	19.7	22	1	1.4×10^8	1.9×10^6	1.4
B. subtilis	19.7	15	18	1.1×10^8	0	0

Microscopic observation of *E. coli* B, YTG-2 S^r, *A. aerogenes*, and *S. marcesens* subjected to a high pressure CO_2 atmosphere heated at 125° or 160° C revealed that nearly 100% lysis had occurred with heating periods as short as 6 h. Cells of *B. subtilis*, however, did not lyse. The 30 min exhaust time used by Molton *et al.* (personal communication) was extended to 1 to 1.5 h in an attempt to reduce lysis. The slower exhaust rate neither increased survival nor reduced lysis significantly.

Since no survival was obtained at high temperatures and as the physical condition of heated cells did not improve with a very slow depressurisation rate, the effect of pressurisation and depressurisation without heating was examined. Cultures of *E. coli* B did not lyse or lose viability when exposed at room temperature (22° C) for 1 h at 9.5 or 19.7 atm CO_2 (Table 1). Exposure for 18 h at 15° C under 19.7 atm CO_2 did not decrease the viability of an *E. coli* B culture either. These results suggest that the pressurisation with CO_2 and subsequent depressurisation were not sufficient to kill unheated cells of *E. coli* B. In two separate experiments with late log-phase cultures of *B. subtilis*, survival decreased to 10.9% and 33.6% after 1 h exposure to 9.5 atm CO_2 at 22° C. At 19.7 atm CO_2, a 1 h exposure of *B. subtilis* cells at 22° C resulted in 0.0032% and 1.4% survival. No survivors were obtained when *B. subtilis* cultures were exposed to 19.7 atm CO_2 for 18 h at 15° C. Thus, with *B. subtilis*, high CO_2 pressure without heating was quite lethal.

In summary, we were unable to reproduce the results reported by Molton *et al.*[1] and suggest that additional studies are needed before conclusions can be reached regarding the thermal protection of bacteria by carbon dioxide at high pressures.

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Production of Bladder Stones by Human T Mycoplasmas

SINCE the first isolation of human T mycoplasmas[1], their pathogenic role has remained controversial. These organisms, present in the urogenital tract of men and women, are unique among mycoplasmas in possessing urease[2]. They have recently been isolated from other mammals[3–5]. Bovine strains have

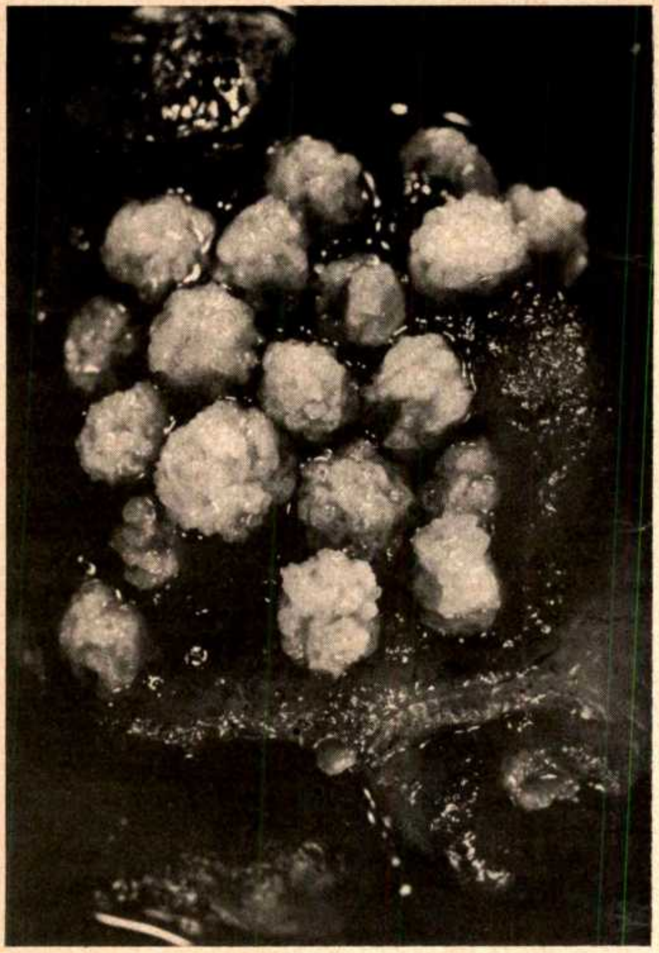

Fig. 1 Bladder stones from a rat killed 6 weeks after renal inoculation with T mycoplasma (×3).

caused experimental pneumonitis in calves[6] and mastitis in cattle. In the latter experiment, human T mycoplasmas were unable to produce mastitis[7]. Human T mycoplasmas have been implicated as causative agents of non-gonococcal urethritis in men and pelvic inflammatory disease, puerperal infection and reproductive failure in women[8]. Their true relationship to human disease, however, remains unknown, and until now they have never been reported to produce disease in experimental animals. We now report the production of bladder stones during experimental infection of rats by human T mycoplasmas.

The T960 strain of human T mycoplasma was grown aerobically at 37° C in trypticase soy broth containing 15% horse serum, 1.25% yeast extract, penicillin (1,000 units ml^{-1}), 0.1% urea and 0.002% phenol red (*p*H 6.0). After overnight culture, an alkaline *p*H change occurred (final *p*H 7.0–7.1). The culture was centrifuged (20,000*g* for 30 min at 4° C) and concentrated 100-fold by resuspending in the supernatant giving 10^6–10^8 colour changing units (CCU) ml^{-1}. Male Sprague-Dawley rats (250–400 g) fed antibiotic-free chow were infected by injecting 0.1 ml into the bladder lumen or renal medulla. They were killed 6 weeks later and examined and cultured for bacteria as previously described[9].

Bladder calculi developed in 78% of male rats after direct renal injection (Table 1). There was no difference in the incidence of stones when 10^6 and 10^8 CCU were used. No bladder stones formed after inoculation with concentrated T mycoplasma broth (*p*H 7.0) alone. The stones were 2–4 mm in diameter (Fig. 1), with up to thirty per rat. Crystallographic analysis showed all the bladder calculi to be essentially pure $MgNH_4PO_4.6H_2O$. All the eleven stones analysed were 100% $MgNH_4PO_4.6H_2O$ except one, which contained 1% carbonate apatite. Ten per cent of normal male and female rats of this strain form minute gravel deep in the renal pelvis. Such gravel was distinctly different from the bladder calculi which develop following T mycoplasma infection, as it was composed of calcium oxalate dihydrate and hydroxyapatite with no trace of $MgNH_4PO_4.6H_2O$. Renal gravel from two rats infected with T mycoplasmas was composed of pure $MgNH_4PO_4.6H_2O$. In none of the experiments with T mycoplasmas was there any effect on the incidence of renal gravel compared with uninfected controls.

Most rats with bladder stones had thickened bladders and dilated ureters. Hydronephrosis was present in one third of the rats. The kidneys showed tiny cortical scars with fibrosis and some round cell infiltration around the needle tract. Similar renal changes were seen following injection of broth only. No bladder thickening, dilated ureters or hydronephrosis was seen, however, in broth controls or rats injected with T mycoplasma which did not develop stones. There was no disease in the prostate or seminal vesicles. Urine, kidney and prostate cultures were negative for bacteria.

Stones formed in three of six rats as early as one week following renal inoculation. Female rats (200–220 g) failed to produce bladder stones (Table 1) and a similar sex difference has been noted in experimesntal tone formation[10]. Intravenous infection failed to produce stones; in males however, inoculation into the bladder lumen of males produced stones at the same high incidence as that seen following renal infection (Table 1).

While attempting to recover T mycoplasmas from the animals, we found a growth inhibitor for T mycoplasmas in renal homogenates similar to that described for large colony mycoplasma[11]. This inhibitor was present at 10^{-1} dilution but was absent on further dilution. No T mycoplasmas were detected in the urine, kidney or prostate of uninoculated rats. T mycoplasmas were isolated from urine and kidney cultures of rats which developed bladder stones following renal infection. A similar isolation rate was seen in infected rats which did not develop stones. Three of ten bladder stones washed extensively in phosphate buffered saline yielded T mycoplasmas on culture.

To determine whether viable organisms are necessary for stone formation, a group of rats was given erythromycin injections daily (80 mg kg^{-1}) for 7 d following renal inoculation with T mycoplasmas. This dose gave urine levels of erythromycin ten times the minimal inhibiting concentration for T960 *in vitro* (3.12 μg ml^{-1}). Erythromycin did not prevent stone formation (Table 1) although urine, kidney and prostate cultures were negative for T mycoplasmas. The implication that viable T mycoplasmas were not required for stone forma-

Table 1 Incidence of Bladder Stone Formation in Rats following Infection with T Mycoplasma

Group	Sex	Route of infection	No. of animals with stones/ Total No. in group
T mycoplasma	♂	Renal	49/63
Uninoculated	♂	Renal	0/17
T mycoplasma broth		Bladder	0/8
T mycoplasma	♀	Renal	0/9
T mycoplasma	♂	Intravenous	0/10
T mycoplasma	♂	Bladder	19/21
T mycoplasma + erythromycin treatment	♂	Renal	7/10
T mycoplasma, acetone killed	♂	Renal	16/20
T mycoplasma 100° C, 15 min	♂	Renal	4/10
P. mirabilis in T mycoplasma broth	♂	Renal	0/5
100° C, 15 min		Bladder	0/5
M. hominis	♂	Bladder	0/12

Rats were all killed 6 weeks after infection. Inocula for these experiments were as follows: T mycoplasma, 10^6–10^8 CCU per 0.1 ml; *P. mirabilis*, 10^9 organisms per 0.1 ml; *M. hominis*, 10^9 CCU per 0.1 ml.

tion was further examined by injecting rats with killed organisms. A culture of T mycoplasmas was centrifuged and resuspended in 33% acetone overnight. The organisms were then centrifuged and resuspended in complete T mycoplasma media (*p*H 7.1) to 0.01 of the original culture volume. Renal inoculation with 0.1 ml mycoplasmas killed in acetone produced bladder stones at the same high rate as with live T mycoplasmas (Table 1). Acetone treatment killed the T mycoplasmas and preserved minimal urease activity as measured by *p*H change. T mycoplasma killed by heat (100° C, 15 min) which had no urease activity still produced stones although at a lower incidence (Table 1) than organisms killed in acetone. Viable T mycoplasmas are thus not required for stone formation.

The observation that heat killed organisms caused stones suggested that T mycoplasmas might do so simply by providing a nidus. This was studied further with *Proteus mirabilis* which has a potent urease and causes $MgNH_4PO_4.6H_2O$ stones during urinary tract infection in rats[12]. *P. mirabilis*, grown in trypticase soy broth was centrifuged and resuspended to 10^9 organisms per 0.1 ml in T mycoplasma broth and then killed by heating at 100° C for 15 min. No bladder stones formed after inoculation of dead *P. mirabilis* (Table 1). The failure of heat killed *P. mirabilis* to cause stones suggests that the nature of the particulate matter may determine whether it can serve as a nidus for calculus formation.

Another mycoplasma species was then examined for stone forming ability to see whether this was a property limited to T mycoplasmas. *Mycoplasma hominis*, the other principal human urogenital mycoplasma, was grown in T mycoplasma media with 1% L-arginine in place of 0.1% urea and the penicillin was omitted. None of twelve rats infected with 10^9 CCU per 0.1 ml developed vesical calculi (Table 1). Stone formation is thus not a general property of all mycoplasma species.

Our results establish the ability of human T mycoplasmas to cause vesical calculi in male rats following bladder or renal inoculation. Stones were not inhibited by antibiotics, and were produced by acetone or heat killed T mycoplasmas so that their formation does not require viable organisms. The specific association of $MgNH_4PO_4.6H_2O$ stones with infection by urease-producing bacteria has long been appreciated[13]. This would suggest that the urease content of T mycoplasmas may be a factor in stone generation. But $MgNH_4PO_4.6H_2O$ stones also occur experimentally in the bladder of rats in the absence of urea-splitting organisms[14].

One of the unresolved aspects of stone formation is the site of nucleation and the subsequent retention of the stone nucleus within the urinary tract[15,16]. The property of T mycoplasma colonies to adsorb mammalian cells[17] may relate directly to this point. For example, T mycoplasmas may attach to epithelial cells in the urinary tract by specific receptors and serve as a stone nidus. As mycoplasmas seem to adsorb serum proteins to their surface[18], T mycoplasmas may also be able to adsorb urinary mucoprotein, the principal component of the organic matrix of stones[19]. Further, aggregation of the Tamm-Horsfall urinary mucoprotein can be caused by lowering the urea concentration[20]. Thus, the common presence of a T mycoplasma in the urinary tract, its capacity to attach to mammalian cells, and perhaps to adsorb proteins to its surface, and aggregate mucoprotein by breaking down urea, all suggest it may serve as a nucleus and part of the organic matrix of human urinary tract stones.

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Genetic Dissection of Active Electrogenesis in *Paramecium aurelia*

NEUROPHYSIOLOGISTS frequently use various drugs and toxins to interfere with specific membrane mechanisms. A genetic approach can achieve the same results. In taking this approach to the problem of excitation it is desirable to have an organism, like *Paramecium*, on which both formal genetic[1] and orthodox electrophysiological[2] studies can be made.

The behaviour of ciliated protozoa is under the direct control of the membrane. Thus, we have efficient methods of screening for membrane mutants which rely on their behavioural peculiarities[3-4]. Wild-type paramecia show "avoiding reactions" to various stimuli[5]. This is accomplished through a period of backward swimming in which the direction of ciliary beat is reversed. This behavioural reaction is correlated with membrane excitation. Mechanical, electrical and chemical stimuli can result in depolarisation of the membrane[6,7]. Suprathreshold depolarisation activates a regenerative increase in Ca conductance. The increase in internal Ca concentration through the Ca influx apparently activates the reversal of ciliary beat[8]. The system returns to normal through repolarisation and active ion transport.

The upstroke of the action potential is considered the result of a Hodgkin cycle, with Ca^{2+} instead of Na^+ carrying the action current in *Paramecium*[6,7]. Concepts such as Ca inactivation, K-delayed rectification, and ion pumping are also introduced to explain the relevant phenomena. Although the molecular mechanisms of these membrane functions in protozoa as in metazoa are still obscure, it is reasonable to think that only macromolecules, presumably membrane proteins, can carry out such complex functions. Genic mutations with corresponding loss of the normal gene products could therefore lead to malfunctions in various membrane processes, which in *Paramecium* are closely associated with the ciliary motion, and could eventually surface as behaviourally aberrant phenotypes.

Kung[3,4,9] and more recently Chang and Kung[10,11] have mutagenised populations of *P. aurelia* with N-methyl-N′-nitro-N-nitrosoguanidine and, by screening, have obtained

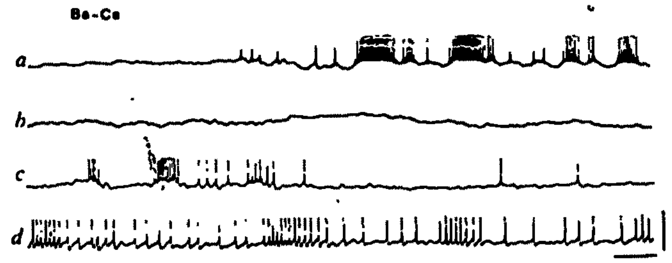

Fig. 1 Potential records of various strains 2–3 min after Ba solution entered the bath. *a*, Wild type; *b*, Pawn; *c*, Fast-2; and *d*, Paranoiac. Calibration: 5 s and 50 mV.

over 170 lines of mutants with various behavioural abnormalities indicating membrane malfunctions. Three unlinked genic mutants each representing a type were used here. These mutants were (1) "Pawns" (Stock d4–95, genotype *pwB pwB*), behaviourally characterised by the inability to reverse their ciliary beat even upon extreme stimulation. This behavioural deficiency was found to be correlated with the loss of proper membrane excitation[12]; (2) "Fast-2" (Stock d4–91, genotype *fna fna*) having an accelerated forward movement and an insensitivity specifically to Na stimulation; and (3) "Paranoiacs" (Stock d4–90, genotype *Pa Pa*) which show a form of violent over-reaction to Na$^+$ but not to other stimuli. The wild type Stock 51s (non-kappa bearing) of syngen 4 of *P. aurelia* was used as control.

Much of the literature on the behaviour of *Paramecium* describes the immediate reactions when cells from one solution are put into a second solution of different ionic composition[13,14]. The bulk of the electrophysiological work in *Paramecium* deals, however, with the evoked potential changes when the cells have been equilibrated for a few minutes to bath solutions of various ionic compositions[6,7]. To study the physiological correlates to chemotactic responses against various cations, we continuously monitored the membrane potential through various changes of ionic solutions.

The cell was first adapted in a K solution of 4 mM KOH, 1 mM CaCl$_2$, 1 mM citric acid and 1 mM Tris-HCl, *p*H 7.2. In this adaptation solution, membranes of wild type and all mutants were quiet and registered a resting potential of -20 to -30 mV.

To test the wild-type membrane reaction to Ba^{2+}, a Ba solution of 4 mM BaCl$_2$, 1 mM CaCl$_2$ and 1 mM Tris-HCl, *p*H 7.2, was let into the bath to replace the K solution. The resting potential gradually diminished to -5 to 0 mV in 3 to 5 min. At this level the membrane spontaneously discharged. Such repetitive firings of all-or-none action potentials are known to be correlated with quick backward jerks observed in the free-swimming animals when they encounter the Ba solution[2].

In reaction to Ba solution, both Fast-2 and Paranoiac mutants generated the Ba spikes typical of the wild type (Fig. 1*a*, *c* and *d*). Considering the variations encountered within the same strain, the frequencies of these spikes observed in different strains are not meaningful. There was, however, a complete absence of action potentials in the case of Pawns treated identically with the other strains. Even under conditions known to increase the spike frequency and duration in wild type, such as a solution with Ca^{2+} concentration reduced to 0.3 mM, Pawn membrane never fired (Fig. 1*b*). This result agrees with the finding of Kung and Eckert[12] that Pawn is not capable of proper electrogenesis due to an impairment of the molecular mechanism for the voltage-sensitive Ca conductance changes. It also agrees with the behavioural observations that all strains discussed avoid Ba except Pawns[3].

We discovered a distinct pattern of active electrogenesis correlated with the chemotactic avoiding reactions against Na$^+$ by the wild type. This pattern appeared in 15 to 25 s after an Na solution (4 mM NaCl, 1 mM CaCl$_3$ and 1 mM Tris-HCl, *p*H 7.2) began to flow into the bath in place of the K solution. No obvious change in the apparent resting level was observed, yet active depolarisations occurred. The rising phase of each episode began with a slow but sudden depolarisation rising from the resting level or rebounding from a slightly hyperpolarised level resulting from a previous episode. The rate of rise increased rapidly to that typical of the upstroke of action potentials. The spike peaked at about 20 mV from the resting level. The potential then oscillated for several hundred ms before returning to or slightly overshooting the resting level.

This gradually damped into patterns with lower spiking frequency, with spikes interspersed with spikeless depolarisations. Careful observation suggested that each spiked depolarisation episode was correlated with a sudden reversal of ciliary beat, and that in free swimming animals it most likely corresponded to the short avoiding reaction observed when the cell encountered Na$^+$. This behavioural correlate, and the fact that 10^{-7} to 10^{-5} g ml^{-1} of tetrodotoxin did not abolish the active electrogenesis, indicated that the action current was probably carried by Ca^{2+} although Na$^+$ may play an important part in events previous to the spikes.

Fewer but active depolarisations were observed when Pawns encountered the Na solution (Fig. 2*b*). Each depolarisation episode differed, however, from that of the wild type (Fig. 2*a*) by a clear lack of spikes. The plateau magnitude and duration of this depolarisation and the kinetics of repolarisation did not differ significantly from the wild-type pattern. This mutated form of active electrogenesis related to no observable behavioural response and apparently caused no ciliary reversal.

No sign of active electrogenesis was observed when Fast-2 first encountered the Na solution (Fig. 2*c*). There was not even the slow rise of resting level found in the other strains in reaction to the Na solution. After a long delay (more than 5 min), however, the membrane eventually drifted to -10 mV. At this time, trains of all-or-none action potentials were observed. This membrane also showed spike-like hyperpolarisations much more frequently than normal membrane.

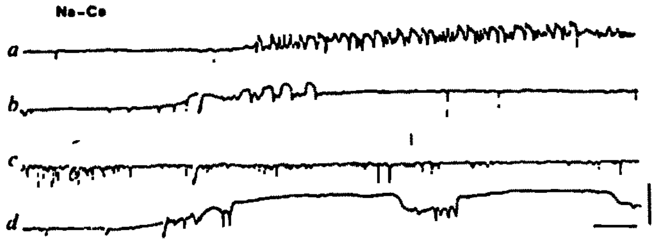

Fig. 2 Intracellular potential records during chemotaxis to Na$^+$. Na solution (without citrate) was let into the bath in place of the adaptation solution at the beginning of traces *a*, *b* and *d*; *c* begins 30 s after the Na solution starts to flow into the bath. Calibration: 5 s and 50 mV.

The membrane of Paranoiac reacted to the Na solution with a peculiar pattern (Fig. 2*d*). There were active depolarisations but they were often sustained at the plateau for up to 60 s. Two such resposes lasting around 20 s are shown in Fig. 2*d*. The kinetics of repolarisation is very different from that of wild type or Pawns. When the plateau phase of the depolarisation was shortened, the recovery kinetics closer to that of the wild type. As in wild type, there was also variation in the upstroke of the depolarisation. The prolonged action depolarisations coincided with the Paranoiac over-reaction to Na of this mutant. Details of the mutant electrical activities will be published elsewhere.

Mutations are usually deleterious, resulting in impairment of orderly biological processes. Thus, the mutant patterns of electrogenesis in the presence of Na^+ could be viewed as modifications of the wild type pattern with single point blockages (Fig. 3).

The depolarisation pattern of Pawns can be viewed as the wild type pattern with a block on the upstroke of the action potential. Current injections of various strengths and durations up to 2×10^{-9} A for 1 s (depolarising the membrane by 20 mV) during our experiments in Na solution evoked no spikes. The nature of the spikeless depolarisation pattern observed in Pawns in reaction to the appearance of Na^+ is obscure. It is apparently triggered by Na^+, never observed in the K-Ca or Ba-Ca solutions used and may relate to chemoreception of Na^+.

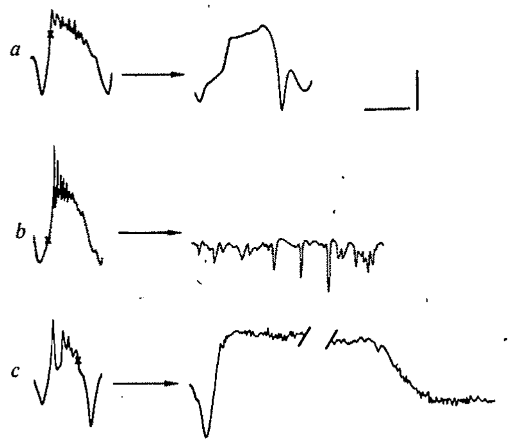

Fig. 3 A hypothesis showing that mutational blockages at various points of a normal depolarisation which occurs during Na chemotaxis can lead to different aberrant patterns. The three figures on the left are variations of the wild type patterns recorded in Fig. 2; the figures on the right are mutant patterns produced by blocks at different stages of a normal depolarisation. *a*, The spiking component is blocked by the *pw* gene leading to the Pawn pattern. *b*, Gene *fna* blocks the early depolarisation event and thus results in the Fast-2 pattern. *c*, Gene *Pa* blocks the proper repolarisation process and thus sustains and prolongs the depolarisation. Calibration: 1 s and 10 mV.

The pattern of Fast-2 in reaction to Na can be viewed as the result of a mutational blockage in the normal depolarisation process, located before the Pawn blockage. This results in a total loss of depolarisation correlating with the specific loss of chemotactic reactions against Na^+. Presence of Ba spikes, delayed action potentials in Na^+ as well as electrically evoked action potentials indicates that the Fast-2 membrane is excitable. The mutational blockage, therefore, does not extend to the component blocked by the Pawn gene.

The intriguing patterns of Paranoiac in the Na solution may be viewed as the result of a genetic block of the normal process of repolarisation. Because of the lack of a proper shut-off mechanism related to Na^+, the membrane potential stays near the peak of depolarisation for a greatly prolonged period and eventually drifts back to the resting level through a secondary repolarisation mechanism unaffected by this mutation.

As a description of the physiological abnormalities of various membrane mutants in *P. aurelia*, this study demonstrates the power of the genetic approach in systematically modifying a complicated biological phenomenon: the active electrogenesis during chemotactic reaction against Na^+. This approach may aid in the search for a molecular mechanism for excitation by correlating the mutant phenotypic expression at various levels of investigation and by identifying the gene products. This rationale of applying genetics to neurobiology was first stated by Benzer[15].

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Regulation of Spawning Behaviour in the Female Goldfish

ALTHOUGH the role of hormones in the control of sexual behaviour in female mammals is well established[1], this is not the case with fish[2]. In spite of several reports that castration of female fish eliminates sexual behaviour[2], only two investigations have demonstrated successful steroid replacement therapy[3,4]. We report here, however, the induction of sexual behaviour in female goldfish (*Carassius auratus*) by injection of ovulated eggs into the ovarian lumen of individuals with vitellogenic ovaries, and also by administration of both eggs and oestrogen to individuals with regressed ovaries.

In goldfish, ovulation can be induced in several days by a change in water temperature from 12° to 20° C. At this 'summer' temperature, females ovulate once or several times within about a month, after which the ovaries regress. At ovulation, the eggs are released into an ovarian cavity which is continuous with the oviduct. Oviposition (release of eggs through the ovipore) occurs during a series of spawning acts. The female approaches and enters floating vegetation in a head-up position, the male following from below. Both turn on their sides and, as they break the water surface, the male presses against the female and appears to push her out of the water. Eggs and sperm are released this time and fertilisation occurs. This procedure may be repeated up to several hundred times during 1–3 h. Spawning behaviour of the female is seen only in the presence of male goldfish and green plants[5].

Encouraged by Yamazaki's[5] findings that (1) spawning behaviour was seen only in goldfish which had ovulated, and (2) hand-stripping of ovulated fish terminated this behaviour, we investigated the role of ovulated eggs. We found that when oviposition is prevented by a plastic and glass plug in the ovipore of an ovulated female, spawning behaviour is extended. Whereas spawning usually lasts less than 2 h, 'plugged' females perform the spawning act (without oviposition) throughout the day of ovulation and sometimes continue the following day.

To determine whether placement of eggs in the ovarian lumen would induce, rather than prolong, spawning, ovulated females were allowed to spawn with a male for 20 min to obtain individual normal spawning rates. The fish were then hand-stripped of all remaining ovulated eggs, placed with an actively courting male, and observed for 1 h to ensure that spawning had terminated. Then all were anaesthetised in MS222 (tricaine methanesulphonate, Sandoz) and given one of three treatments: (1) a handling control, (2) a plastic and glass oviduct plug fitted in the ovipore, and (3) an injection of the individual's own ovulated eggs through the ovipore, followed by an ovipore plug to prevent egg loss. In this last treatment, eggs were squeezed into a plastic cup and taken up in a 1 ml plastic syringe fitted with plastic tubing (PE 190). Eggs were injected into the ovarian lumen by inserting the tip of the plastic tube through the ovipore. It was important to prevent the eggs coming into contact with water, as this caused adhesion and hardening.

Within 10 min of treatment each female was placed with a sexually active male and behaviour recorded for 1 h. There was no difference between pretreatment spawning rates of the three groups, but injection of eggs stimulated spawning (Table 1).

Table 1 Effects of Injection of Ovulated Eggs into the Ovarian Lumen on Spawning Behaviour of Ovulated, Hand-stripped Goldfish

Group	N	Before treatment Spawning rate* No. spawning	Mean	Range	After treatment Induced spawning rate† No. spawning	Mean	Range
(1) Control	11	11	32	14–60	1	7	
(2) Oviduct plug	11	11	40	20–56	4	6.5	1–11
(3) Egg injected	11	11	34	9–60	11	28	7–57

* No. of spawning acts per 20 min.
† No. of spawning acts in the most active 20 min of the test hour.

Next, we sought a time after ovulation when egg injection did not induce spawning. Surprisingly, injection of ovulated eggs into females which had not ovulated for at least a month usually resulted in spawning within 2 h. Furthermore, physical substitutes for eggs (gelatine, petroleum jelly and ovipore plugs) sometimes induced spawning, but the response was much smaller than with egg injections, suggesting that chemical as well as mechanical stimuli may be involved.

Females kept at 20°–22° C for 4 months or longer invariably failed to respond to injected eggs. Their ovaries were small, resembling those of hypophysectomised fish, and probably represented the phenomenon of temperature regression found in *Gillichthys*[6]. The oocytes were in the first growth phase with little or no yolk.

We thought that the unresponsiveness of temperature-regressed fish may result from reduced oestrogen secretion. To test this, we investigated whether 17β-oestradiol would restore the spawning behaviour of these fish. Four groups of female fish were used. Group 1 (control) received four or five saline injections on alternate days before being injected with eggs on the test day. Group 2 received oestrogen but was not given egg injections. Group 3 fish were injected with oestrogen before receiving eggs. Group 4 (recently-ovulated control) received only egg injections on the test day.

Fish in groups 1, 2 and 3 had regressed ovaries. In each group, half the females were known to have ovulated soon after being warmed to 20° C several months earlier; the others had not ovulated in that period. Fish of group 4 had ovulated 3–9 d before the test day. 17β-Oestradiol was injected intraperitoneally as a saline suspension (0.6% NaCl, four drops Tween 80 per 100 ml) at a concentration of 2.5 μg μl^{-1} and a dose of 20 μg per g body weight on alternate days for 7 or 9 d. Control fish received either an equivalent volume of saline and Tween 80 (group 1) or no previous treatment (group 4). On the morning of the day after the final injection, females in groups 1, 3 and 4 were anaesthetised in MS222, injected with eggs (0.025 ml per g body weight) from an ovulated female, fitted with an ovipore plug, and placed in a 15 gallon test aquarium at 20° C. Group 2 was simply anaesthetised and left to recover in the test aquarium. An actively courting male was added to the aquarium after 30–50 min and the pairs were observed continuously for 3.5 h.

Table 2 Effect of 17β-Oestradiol on Spawning Behaviour of Temperature-regressed, Egg-injected Female Goldfish

Group ovaries	Treatment	N	No. spawning	Mean spawning acts per 3 h*
(1) Regressed	Saline and eggs	12	2	63
(2) Regressed	Oestradiol	6	0	0
(3) Regressed	Oestradiol and eggs	12	12	66
(4) Active	Eggs	7	7	94

* Based on responding individuals.

Of twelve control fish treated with saline and eggs, only two spawned (Table 2: group 1). The response was not measurably affected by whether the fish had ovulated since being held at 20° C (one of six spawned in each case). Oestradiol alone failed to induce spawning in the regressed females in group 2, again demonstrating the necessity of having eggs in the ovarian lumen. In contrast, spawning was observed in all regressed fish receiving oestradiol and eggs (group 3) and in all recently ovulated fish receiving only eggs (group 4). Our method for obtaining regressed female goldfish is, however, crude: some fish are not fully regressed and have ovaries in early stages of vitellogenesis. The two responding fish in group 1 were in this condition, suggesting that their spawning was due to endogenous steroids.

Yamazaki's[5] and our own observations suggest that under natural conditions female goldfish have an extended breeding season during which they may ovulate and spawn several times during a period of weeks. An important role of oestrogen seems to be to induce and maintain responsiveness to the stimulus of ovulated eggs; there is no indication whether oestrogen acts peripherally or centrally, or both. The fact that readiness to perform oviposition behaviour in response to male courtship is induced by egg injection up to a month after the last ovulation indicates that the female goldfish is in an endocrine state appropriate for breeding for an extended period, but that spawning is normally synchronised with ovulation by the stimulus of a large intra-ovarian mass of ovulated eggs, which acts as a fine control in the timing of sexual responsiveness.

It would be interesting to determine whether comparable mechanisms exist in other fish or in other egg laying lower vertebrates. The fact that oestrogen alone restores sexual receptivity of female guppies which have been both hypophysectomised and gonadectomised[4] suggests that in viviparous forms stimuli from the gonads or accessory structures may not be essential for the expression of sexual behaviour. In previous studies[2], the failure to induce sexual behaviour in female fish by oestrogen therapy may have been due to the absence of the requisite stimuli from internal sexual structures.

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Evidence for the Two-state Model of Nematode Behaviour

A MODEL for the swimming and orientation of *Panagrellus silusiae* based on transitions between two behavioural states has been proposed[1]. The basis of this model is that in normal, unstimulated conditions the nematode follows a more or less linear path with the anterior and posterior ends of the animal sweeping through a minimal area. This is termed the 'normal' behavioural state. When stimulated by chemical or physical means, the animal enters the 'activated' state, making rapid changes in orientation with the ends of the animal sweeping through a maximal area. In the case of a gradient of an attractant, the animal remains in the activated state until it orientates to the gradient by sensing the maximum difference in intensity of the stimulus between the posterior and anterior sensory structures, at which point the animal returns to the normal behavioural state and migrates to the source of attractant. Laser microbeam studies indicated that the copulatory spicules of the free-living *P. silusiae* function as the posterior receptors for orientation to mating attraction in this species[1], and a sensory function has also been found for the spicules in other species[2].

Ward[3] proposed that chemotaxis of the free-living *Caenorhabditis elegans* is mediated by comparisons of stimuli by the anterior sensory receptors at successive lateral sweeps (klinotaxis). This mechanism requires some type of short-term memory system in nematodes, which has been demonstrated in *P. silusiae*[1].

The 'two-state' model was based on frame-by-frame analysis of video recordings of *P. silusiae* swimming in response to a range of chemical and physical stimuli, but the klinotaxis model[3] was based on examination of the tracks of normal and mutant nematodes in response to gradients of cyclic nucleotides, ions, amino acids and *p*H. Here we report further support for the two-state model based on analysis of tracks of *P. redivivus* males migrating in gradients of sexual attractant.

Tracks of individual males of *P. redivivus*, strain N, on 4% Czapek dox agar were recorded photographically (N. Croll, personal communication). The sources of mating attraction were blocks of 1.5% agar exposed to females for 24 h (ref. 4). Males were placed 2.5 cm from the source of attractant and tracks were recorded after 15 min. Analysis of the tracks involved measuring the linear distance travelled, the number of turns (changes in direction of tracks), loops (360° turns accomplished through forward motion only) and reversals (backward motion) made in the 15 min period (Table 1).

Table 1 Activities of Adult Males of Strain N *P. redivivus* in Unstimulated Conditions and in Response to Mating Attractant from Females of Strains C and N

Stimulus	Distance (cm)	Loops (No.)	Reversals (No.)	Turns (No.)
None	1.71±1.06	5.43±1.61	22.81±3.16	26.59±4.44
C females	2.13±0.19	16.59±3.31	34.42±3.96	128.79±14.47
N females	2.36±0.42	15.30±3.83	47.00±9.44	150.00±29.86

Figures are means during a 15 min test period.

Movement to attractant involves no significant increase in the distance travelled but does show significant increases in the number of loops, reversals and turns relative to those of males moving without attractant. This shows that the behavioural activities of males responding to sex attraction are different from the normal behaviour. The pattern of the tracks of males responding to sex attractant (Fig. 1*a*) is exactly what would be predicted by the two-state model.

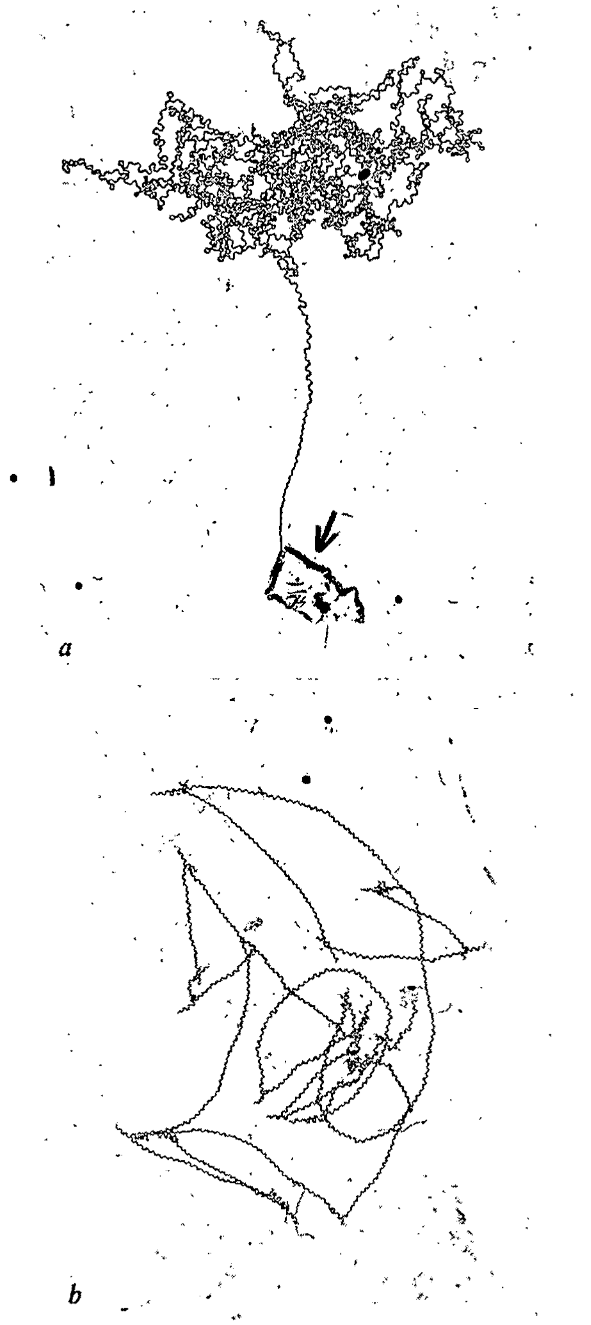

Fig. 1 Tracks of *P. redivivus* males, *a*, in response to sex attractant, *b*, unstimulated. The male responding to attractant (arrow) first undergoes a period of rapid changes in orientation (activated behaviour) and then migrates directly to the source of attractant.

Initially the males make rapid, constant changes in their orientation, with increased turning, looping and reversal; this is the period of activated behaviour. As the animal migrates towards the attractant the path is linear and direct; this is normal behaviour. Males moving when no attractant is present show a more or less linear pattern (Fig. 1*b*) broken by widely spaced changes in direction.

The two-state mechanism for sexual attraction does not preclude the functioning of klinotaxis in other classes of nematode chemotaxis. In *P. silusiae* sexual attraction is limited to one stage of the life cycle of one sex[4], but non-specific food attraction occurs at all stages of both sexes. This latter attraction does not involve the two-state mechanism.

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Calcium Carbonate in Termite Mounds

The presence of appreciable quantities of calcium carbonate in termite mounds on non-calcareous soil has intrigued pedologists for many years. Milne[6], for example, found a termite mound with 7% calcium carbonate and estimated that it contained about 2 t of calcium carbonate excluding the hard limestone (53% $CaCO_3$) base of the mound. The soil below the base of a termite mound may also be calcareous. The soil underneath one termite mound in an area of non-calcareous soil was found to have a mean of 1.7% calcium carbonate to a depth of 6 m, or about 20 t of calcium carbonate[9].

The task of finding the primary methods of calcium carbonate accumulation has been hindered by the fact that termite mounds are complex systems involving biological and physical processes. Milne[6] and Pendleton[7] suggested that the most probable biological method was the collection of food by termites and formation of calcium carbonate from the mineralised residues. Other biological methods of calcium carbonate accumulation that have been advanced are as follows: (1) exchangeable calcium in soil collected incidentally by termites with their food[6]; (2) exchangeable calcium in soil collected purposely by termites requiring an alkaline environment[8]; (3) calcareous material collected from below the depth of soil said to be non-calcareous[3,7]; and (4) calcium-containing groundwater brought up by termites[2]. The physical methods proposed all involve evaporation of water containing calcium bicarbonate from a termite mound as the means of accumulating calcium carbonate, but they differ in the source and mode of entry of water into the mound. Thus Milne[6] suggested that water moves upward into the termite mound by capillary action. Den Doop[4] considered that the water evaporating from the mound was derived from surrounding land in the wet season. Boyer[2] too describes how water from perched water tables drains laterally and collects underneath mounds of *Macrotermes subhyalinus*. Finally, Hesse[5] found an association between calcareous *Macrotermes* mounds and poorly drained soil which led him to state that saturation of the base of a termite mound by groundwater was a prerequisite of calcium carbonate accumulation.

The early ideas on the mode of accumulation of calcium carbonate in termite mounds were considered by Pendleton[7] who said of some of them that they "seem too fantastic to repeat". He came to the conclusion that there was no known method by which termites or pedological processes could bring about the observed accumulation of calcium carbonate in termite mounds. There seems therefore to be a need for a different approach to the problem. A weakness of all proposals made hitherto is the long time that it would take to accumulate large amounts of calcium carbonate.

I have constructed a hypothesis that could account for more rapid accumulation of calcium carbonate in a termite mound.

The hypothesis (Fig. 1) is that calcium carbonate can accumulate in termite mounds by means of a two-stage process.

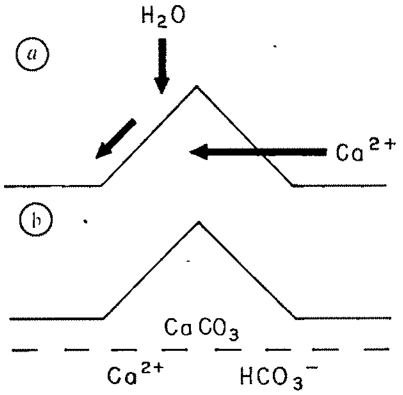

Fig. 1 Two stages in the accumulation of calcium carbonate in a termite mound. *a*, Elevation of the *p*H of the termite mound. This is brought about by termites carrying vegetation into a mound, aided by the fact that a mound sheds rainwater; *b*, precipitation of calcium carbonate as a result of contact between a termite mound of high *p*H and groundwater of low pH_c.

(1) Elevation of the *p*H status of the termite mound above that of the pH_c of the groundwater. An increase in the *p*H of a mound is brought about by termites importing vegetation which is decomposed to release exchangeable bases. Retention of exchangeable bases in the termite mound is facilitated by a low degree of leaching because a mound sheds water[9].

(2) Saturation of the base of the termite mound by groundwater containing calcium bicarbonate which precipitates as calcium carbonate according to the equation

$$Ca^{2+} + HCO^-_3 \longrightarrow CaCO_3 \downarrow + CO_2 \uparrow$$

The tendency for calcium carbonate to precipitate from irrigation water on contact with soil is a well known alkalinity hazard of irrigation schemes, and it has been found by Bower[1] to be related to a modified Langelier saturation index:

$$\text{Modified saturation index} = pH_s - pH_c$$

where pH_s is the *p*H value of the soil and pH_c is the theoretical *p*H value that the water would have if in equilibrium with calcium carbonate. Bower states that precipitation of calcium carbonate occurs if the index is positive. Moreover, the percentage of the applied bicarbonate that precipitated was found to be highly correlated with the value of the index.

This *p*H-dependent precipitation of calcium is related to the presence of bicarbonate and carbonate ions and does not occur with other common anions in groundwater, namely, chloride and sulphate. Formation of calcium carbonate in termite mounds in some regions and not in others may therefore, perhaps, be explained by differences in the anion composition of the groundwaters.

The rate of accumulation of calcium carbonate by means of the *p*H-bicarbonate process outlined here could be relatively fast in situations where groundwater of low pH_c flows past the base of a *Macrotermes* mound of high *p*H value. Consequently, if this hypothesis is correct, it will be unnecessary to assume that termite mounds have existed for the thousands of years that it would take to accumulate large amounts of calcium carbonate by evaporation of water.

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book reviews

New horizons

Cosmology Now. Edited by Laurie John. Pp. 168. 8 plates. (BBC: London, November 1973.) £2.75.

THIS is, without doubt, the best introduction to modern cosmology available in book form. The list of contributors, each of whom provides one or two chapters, reads like a role of honour of the young cosmologists who have been at the forefront of the revolution in the subject over the past twenty years, with the addition of a couple of 'grand old men' such as Professor W. H. McCrea (whose outlook is certainly as youthful as that of any of the other contributors). For once, a claim made in the cover blurb (quoting Sir Bernard Lovell's introduction) can be taken at face value: this really is "the best description of the universe which can be given today".

But lest it seem that I am in the pay of the book's editor I should, perhaps, elaborate on the areas where it falls short of perfection, in the hope that some of these shortcomings might be rectified in a later edition. There is one glaring omission—in a book which is sure to attract the casual reader, and to whet his appetite for more cosmological fare, there is no bibliography or guide to further reading. Compared to this, the other points to which I take exception are only minor irritations, largely due, it would seem, to the editor's unfamiliarity with astronomy. Laurie John produced the radio series from which the book derives, and no doubt it is fitting that he should provide the introductory paragraphs which head each chapter. But surely among the wealth of cosmological talent contributing to the book someone could have checked these paragraphs, and the captions to the illustrations, for astronomical howlers?

Reference to quasars as "star like objects that may lie near the edge of the universe" is certainly misleading without qualification; far worse is the caption to Plate 8: "The star arrowed is a quasar"! It also seems a shame to introduce Martin Rees solely as "Plumian Professor of Astronomy" when the rest of his title ("and Experimental Philosophy") relates so appositely to the content of the book in general and his own contribution in particular. Apart from the quote from Sir Bernard Lovell the cover blurb is irritatingly out of key with the book, dragging in a reference to "the 'black hole', that ever-growing celestial maelstrom" which is likely to cause further distress to those astronomers already infuriated by the extravagant popularisations of black hole astronomy which have appeared recently; they can, however, safely ignore the words on the cover and be guided as to the true nature of the book by Professor Roger Penrose, who points out that no astronaut is likely to encounter a black hole by accident—"they would have to seek out a black hole deliberately if they wished to experience this 'ultimate trip' ". My final objection is purely a personal one—I have no liking for Professor John Taylor's homespun cosmological philosophy. But that may indicate a failing in me rather than in him.

Such a small catalogue of complaints shows, perhaps, how good the book is in general. As far as the detailed exposition of cosmological ideas is concerned there is little to say here except that all the contributions are readable and intelligible without any prior mathematical knowledge, and the few illustrations are equally clear and provide a valuable addition to the text. Quite apart from the 'introductory text' aspect of this volume, however, the contributions provide a valuable insight into the personalities of the contributors and the kind of bric-a-brac of odd bits of information which make it valuable in historical terms. I was particularly intrigued, for example, to discover from Dennis Sciama's contribution that Dicke and his colleagues were unaware of Gamow's earlier 'hot big bang' model of the Universe when they reintroduced the idea in 1965; I would have thought every astronomer remembered Gamow's work, if only because of his cunning choice of co-authors in writing the classic paper by Alpher, Bethe and Gamow—certainly I knew of this work in 1965, when much to my delight I discovered that paper as part of an undergraduate project. And certainly once discovered it is never forgotten.

Professor J. V. Narlikar also produces a memorable sentence: "Until the observational situation clarifies further it is premature to write off the steady state theory". That is particularly encouraging for those, like myself, who have a fondness for the steady state theory, and it does seem that the model has regained some ground since the dark days of the mid-1960s. But I must confess that while my gut reaction is still 'steady state, right or wrong' my head tells me that the evidence in favour of the hot big bang is very nearly overwhelming.

I also have a fondness for Mach's Principle, first propounded by Bishop Berkeley and today, it seems, rather more widely approved than steady state cosmologies. And when investigating the triumph of head over gut in the big bang–steady state dilemma, the future historian of science will no doubt be intrigued to learn from Sciama's contribution that he was persuaded, in 1966, of the great difficulties presented by trying to explain radio observations in steady state theory terms by "my student, Martin Rees". That same Martin Rees contributes to the book in his capacity now as Plumian Professor; an indication of just how much ground has been covered by essentially one generation of cosmologists.

So the book is well suited to the casual reader, to the historian of science, to specialist astronomers and to students. With an eye particularly to the needs of the latter, I hope that it may soon be available in paperback form at an even lower price, although it is certainly good value already.

JOHN GRIBBIN

Descriptive embryology

Embryology and Phylogeny in Annelids and Arthropods. By D. T. Anderson. Pp. xiv+495. (Pergamon: Oxford and New York, October 1973.) £8.

THE human brain, it would seem, is well adapted to the recognition and analysis of two-dimensional patterns, corresponding to retinal images, but is, as all teachers of anatomy know, far less adept at dealing with three-dimensional configurations. In embryonic development we are confronted with three-dimensional patterns changing in time; to produce a total image of such a development as a whole would be equivalent to thinking in four dimensions. To go even beyond this, in seeking to analyse a comparison between the embryonic developments of a whole series of animal species would in effect add even more dimensions to the problem. The daunting difficulty of this goes far to explain the recent unpopularity of studies in comparative descriptive embryology. Professor Anderson's book is to be welcomed as a bold and substantial inroad on this difficult field, comparable with Dawydoff's (1928) *Traité d'Embryologie Comparée des Invertebrés* or Johannsen and Butt's (1941) *Embryology of Insects and Myriapods.* The work is not merely a compilation from published sources, but incorporates much original work of Professor Anderson.

In seeking to draw phylogenetic conclusions from studies of embryology, the older authors looked for elements of Haeckelian "recapitulation", that is embryonic features resembling those of adults of more or less remote ancestral forms. Many features described by Anderson might be considered as recapitulatory in this sense; for example, the pre-mandibular somites and appendage rudiments of insect embryos, the transient coelomic sacs of numerous Arthropod embryos, the trilobite-like features of Crustacean nauplii and of the newly hatched *Limulus,* and the widely separated paired primordia of the insect labium. Most recent embryologists, Professor Anderson among them, minimise or ignore this line of evidence for ancestry.

Discounting recapitulation, he tries to draw phylogenetic conclusions from comparisons of overall developmental patterns in different taxa. In practice, of course, the limitations of humanity oblige him to concentrate attention on certain features abstracted from the total process; cleavage patterns, formation of germ layers, somite differentiation, are discussed in particular detail. Much stress is laid on 'fate maps' in relation to early embryonic stages. In this connection no distinction is made between 'mosaic' types of development, as in typical spiral cleavage of Annelida, where the future development of every cell is fixed from the beginning of cleavage, and 'regulation' types, known, for example, in many insects, where for a considerable time pieces detached from the embryo can be made good from the rest of it. The book gives well illustrated accounts of typical developmental patterns of all the major groups of Annelida and Arthropoda, but several phylogenetically interesting minor ones, such as the Echiuroidea (whose true position is probably in Annelida), the Tardigrada and Pentastomida, Myzostomida and Pycnogonida, for which embryological information is available, are omitted altogether from consideration.

The phylogenetic conclusions drawn by Professor Anderson coincide almost exactly with those expressed in a recent paper by Dr S. M. Manton "Arthropod Phylogeny—A Modern Synthesis" (*J. Zool., Lond.,* **171**, 111; 1973), to whom Anderson pays handsome tribute and dedicates his book. Dr Manton in turn frequently cites Anderson in support of her own, more dogmatically expressed views. A central conclusion of both authors is thus expressed by Manton: "It used to be supposed that the Arthropoda were monophyletic . . . now the polyphyletic nature of the group is inescapable, as is the acceptance of parallel evolution of roughly similar structures, for instance, compound eyes and biting jaws, in unrelated groups, such as insects and crabs". Dr Manton splits the Arthropoda into three groups, Uniramia (Onychophora, Myriapoda, Hexapoda), Crustacea and Chelicerata, which she treats as independent phyla; Anderson goes even further in questioning whether these three groups could even have had a common ancestor within Annelida. Dr Manton consigns the Trilobita to a kind of limbo outside her system, remarking also that "We can do no fossil embryology". If embryology is restricted to development within the egg, this may be true, but Anderson devotes a good deal of space to post-hatching stages in Polychaeta and Crustacea, and the palaeontologists have traced developmental stages in Trilobita back to a stage apparently comparable with a nauplius. The "modern synthesis" completely ignores evidence from recent protein studies, for example, of lactic dehydrogenases and similar enzymes, or from moulting hormones and other such substances.

It seems likely to me that future evidence will offer little support to the phylogenetic ideas of either author; Professor Anderson's book will, however, retain its value for embryologists, on account of the good and detailed accounts it provides of the earlier development of such a large and important group of animals, as well as for the excellent, full and up to date bibliographies which are appended to each of his chapters. If Dr Manton's phylogenetic theories are eventually proved wrong, this will not detract from the value of her earlier work on the movements and coordination of arthropod appendages, and her latest paper should at least act as an intellectual challenge to those who disagree with her.

R. A. CROWSON

Salomon's house

Science and Politics. By Jean-Jacques Salomon. Translated from the French by Noel Lindsay. Pp. xxii+277. (Macmillan: London and Basingstoke, July 1973.) £5.50.

THE science policy concerns of the 1960s are here paraded in full academic robes. The author was a Professor of Philosophy in Paris before he became head of the Science Policy Division of the Organisation for Economic Cooperation and Development, and his approach is that of a scholar: he gives no quick tips on how to provide the manpower, organise the research institutes or run an international project, but he is well read and his views are always interesting and worth considering.

Tackling his subject historically, he sees science policy as "the child of war", the military model being the ideal one for the mobilisation of science. When he discusses policy for science, he never tires of denying that the rationality of science somehow rubs off on scientific affairs, including technological forecasting. For readers in England he need not have repeated the point so often—the danger of a take-over by quantitative methods has never been great here, partly perhaps because of the healthy innumeracy of our decision-makers. Alvin Weinberg's criteria for scientific choice and the associated debate in *Minerva* get the space they deserve on account of their intellectual interest as well as the criticism they deserve for being "non-operational".

On the matter of science in policy, Salomon rejects the notion of an apolitical elite and insists, with R. Gilpin, that it is impossible for scientists to free themselves from values and non-technical assumptions. He introduces the term "technonature" to denote the borderland between science and government, between knowledge and power; it is "the inevitable meeting ground between the ideology and the scienticity of science" and it "brutally teaches scientists that informing political decision is not the same thing as forming it".

There is an interesting historical inaccuracy regarding the author's eponymic house. In Salomon's house as conceived by Francis Bacon in *New Atlantis,* the last of the nine categories of workers, the Interpreters of Nature, "raise the former discoveries by experiments into greater observations, axioms and aphorisms". J.-J. Salomon says their function is "turning experiment into action" (page 8). Trivial in itself, the shift from axioms to action is indicative of one of his leading ideas: that one can "no longer" separate pure from applied research, theory from practice. But when in history was there a time when science was "as innocent and pure as on the first day"? Is this anything more than another Adam-and-Eve myth? Galileo, it should be remembered, made money by selling a "military compass".

Salomon seems to suggest that science lost its innocence around the time of the Second World War—as though the birthright of the Mertonian norms (disinterestedness and so on) was then sold for a mess of pottage. This view is more convincing as a description of ontogeny than of phylogeny; it applies quite clearly to individual scientists such as Oppenheimer but much less clearly to science as a whole. Salomon even implies (page 183) that competition for recognition has become intense only since the 1920s. I suspect he misattributes the historical transformation: it is not so much the character of science which has changed as the writing about science which has become more sophisticated and realistic.

He is not critical enough of the discovery push type of thinking which sees technological innovation as the result of pure research. Historians—even non-Marxist ones—have realised that it is at least as illuminating to analyse the technological roots of science as the scientific roots of technology, and work on modern industrial innovation indicates that today, too, need pull is more important than discovery push and that the inputs from science into technology are in fact rather tenuous.

By the time a book appears in a second language, three years after its original publication, it should be free of historical howlers such as the one about "the steam engine, of which Watt had the first idea as early as 1765" (page 27). Readers used to the down-to-earth directness of the best English writings on the subject may find that too many of the Franco-English sentences need rereading before they yield a meaning.

F. R. JEVONS

Making the best of it

Optimum Structures. By W. S. Hemp. Pp. xii+123. (Clarendon: Oxford; Oxford University: London, May 1973.) £7.50 boards; £3.50 paper covers.

IN recent years a great deal of attention has been focused on optimal or optimum structures. Such systems have their components so arranged as to minimise internal forces and so dimensioned as to maintain uniform stresses. Originally this field of study proved to be of special interest to aircraft designers, but recently civil and structural engineers have also turned their attention to it. The basic principle of optimisation applies to all classes of structures, and the recent trend towards improved design of structures of minimum weight obtained at a minimum cost gathers momentum.

To achieve these aims a detailed knowledge of various factors and a clear picture of their mutual interdependence are required. Optimisation can produce the desired result and it can be described as a procedure used by the designer to decide on the one specific solution in a defined set of possible alternatives that will best satisfy a selected criterion. In the past the optimisation of structures has required lengthy and time-consuming calculations. The advent of digital computers is changing this, enabling the designer to compare many solutions in his search for the optimum structure. At the same time the interest grows in the fundamental philosophy regarding optimum structures.

Although there are already several papers published on this topic, Professor Hemp's book is unique, not only because it deals at great length with the basic principles of the analysis of optimum structures, but also because it incorporates a great deal of fundamental and highly original work of the author. Whereas most of the published articles deal with numerical methods, this book is mainly concerned with the derivation of the general principles of optimum structures using linear programming and the calculus of variations. It covers the treatment of pin-jointed frameworks, beams, circular sandwich plates, Michell's structural continua and plates loaded in their planes. Optimisation of structures can very easily become highly sophisticated and extremely difficult depending on the number of constraints or independent parameters considered in the analysis.

Although the treatment by nature is highly mathematical, the author has succeeded in producing a lucid exposition of the up-to-date methods of determining structures of least volume of material. He pays special attention to the use of Lagrangian multipliers. This is a book which many research workers and some designers will find extremely valuable in their search for the ideal structural form.

Z. S. MAKOWSKI

Currents in insulators

The Theory of Electrical Conduction and Breakdown in Solid Dielectrics. By J. J. O'Dwyer. (Monographs on the Physics and Chemistry of Materials.) Pp. ix+317. (Clarendon: Oxford; Oxford University: London, September 1973.) £10.

ONE might think that the subject of 'currents in insulators' was a contradiction in terms. This is not so: insulators have a wide forbidden band and hence often block the transport of current. But while it takes effort to introduce carriers into the conduction band, once they are there they run down potential slopes in the conventional way unless arrested by imperfections. Because of the difficulties of experimental study, the theory of charge transport in dielectrics is not so advanced as in semiconductors and metals. Injection of charge into thin-film insulators is, however, now seen to be potentially useful and this should raise the level of research performed.

The other technologically relevant field is the one treated in this book: when insulators are stressed to a very high field (over 10^8 V m^{-1}), new phenomena occur which turn the material into a good conductor, though perhaps only over a limited part of the area exposed to stress. This part then carries an increasing current until the material overheats and is destroyed. In high-voltage technology, these effects must be minimised where possible. For this, one must understand the conduction phenomena occurring at the onset of breakdown. The object of this book is to lay out, in a concise manner, the various pieces of theory and research concerning conduction in insulators which aid this understanding and then to build on these concepts a picture of the complex events leading to the final catastrophic breakdown.

The author has, on the whole, achieved these limited aims with a commendable economy of space. After a useful initial survey chapter, which sets the scene well, the reader is led, with little deviation, through the several types of conduction possible in insulators. The research results of many workers are quoted for cases in which it is advisable to show how a particular mathematical model is embodied in real life. So far, so good; however, the author is a specialist in the field of breakdown phenomena (this book is actually a greatly broadened form of a 1964 book outlining his own theory of breakdown). Perhaps because of this, he seems to lean over backwards to be impartial and, as a result, withholds any overt judgments on the relative merits of the experiments and theories described. Not only does he seem to be saying "now judge for yourself", but one is also left with the erroneous impression that every model ever devised for conduction in insulators is equally true and common, even though some do, in fact, conflict. It is a pity that the benefits of a highly informed point of view were so clearly withheld.

There is thus little to disagree with in this book. It is a good, dispassionate record of published fact. A few omissions can, however, be sensed by scanning the author and subject indexes; there is no entry for 'photoinjection' or 'filamentary conduction', although both loom large in charge transport through thin-film insulators—a field which one would expect to be covered in a book of the aims stated. Neither are the investigations of Berglund, Powell or Nicollian on various types of injection into silicon dioxide films, or the work of Dearnaley or Harrop on filamentary and a.c. conduction phenomena, given any mention. In general, the book thus has a bias towards 'bulk' experiments and places little reliance on thin films as media for elucidating transport phenomena—an unfortunate blind spot, since Lynch has recently built on such work to predict successfully the breakdown strength of silicon dioxide.

With this exception, it must be said that for a research student or working scientist who wishes to be quickly and smoothly introduced to or updated with respect to the concepts of transport in insulators (ionic as well as electronic), it will be well worth his while to have this book on his shelf. A lot of effort has been expended in whittling down the arguments to their core and selecting the best experimental examples for the reader's inspection. If the reader then has to go on a bit further in order to make his own value judgments, he will still have saved himself a lot of time in the library. A. G. HOLMES-SIEDLE

Fermi surfaces

The Fermi Surface: Its Concept, Determination, and Use in the Physics of Metals. By A. P. Cracknell and K. C. Wong. Pp. xii+565. (Clarendon: Oxford; Oxford University: London, August 1973.) £15.

IN his original work in 1926 Fermi introduced the thermodynamic potential, or degeneracy temperature—what we now call Fermi level—of a gas of electrons. Though he was well aware that these ideas were relevant to electrons in metals, he could hardly have foreseen what central role this concept would assume in solid-state physics and particularly in the theory of metals. As the preface to this book reminds us, Professor Mackintosh thinks that the most appropriate definition of a metal is "a solid with a Fermi surface", the surface being the locus of points in momentum space at which the one-electron energy is equal to the Fermi level. This quantum concept, in conjunction with the consequences of crystal periodicity, provides the foundation on which the properties of metals can best be discussed. The beautiful, and sometimes bizarre, shapes of these surfaces (the monster, the coronet, the super-egg . . .) are natural examples of abstract art, almost reflecting unconscious archetypal models.

The work which has led to the determination of these surfaces is among the most ingenious, elegant and fascinating in modern physics and this book aims at giving a complete authoritative account of all its aspects. It is a combination of an introductory treatise and an extended review article. The first two chapters give those parts of band theory which are necessary for a proper understanding of both theoretical and experimental methods. Chapter 3 describes the experimental methods, then two chapters, bringing up to date the well known review articles by one of the authors, describe the Fermi surfaces of metallic elements. Chapter 6 describes in an elementary way the effect on Fermi surfaces of electron interactions, and the work ends with an admittedly perfunctory treatment of alloys.

Probably the most striking characteristic of this exposition is its extreme clarity. The treatment of even difficult points flows effortlessly and should be well within the grasp of graduates who know the fundamentals of solid-state physics and of quantum theory (old style—the Dirac notation is not used). Of course the task of giving a simplified treatment which is not partly misleading is an impossible one, but we come near to achieving it here, and many teachers will get valuable hints for their lectures. By and large the choice of material appears very sensible; all the necessary definitions and explanations are given (or almost all: for instance the definition of Clebsch-Gordan coefficients seems to be missing); the usual "it can be proved that" is always coupled to an appropriate reference. Some sections are perhaps unduly discursive and some elementary items are included which could have been assumed to form part of the background, for example, Einstein's and Debye's theories of lattice specific heats. A notable omission is any reference to liquid metals. At least the extent of blurring of the Fermi surface might have been indicated, and a reference to Professor March's monograph given.

The book is thus a very useful introduction to the subject, but its main value is as a collection of results of work on Fermi surfaces complete up to 1971 with some later references. The total number of references is almost one thousand and they all fit neatly into their appropriate slot. I have failed to find any aspect of any importance which is not adequately covered. The authors will receive the heartfelt gratitude of all "Fermiologists" and it is safe to say that this will remain the standard treatise on Fermi surfaces for a long time, even if some parts should become surpassed. It is a pity that the price should be so high, even taking into account today's wild inflation, and the quality of reproduction of the illustrations is not outstanding.

L. PINCHERLE

Gas theory

Mathematical Theory of Transport Processes in Gases. By J. G. Ferziger and H. G. Kaper. Pp. xiii+579. (North-Holland: Amsterdam and London, 1972.) Dfl. 120; $35.25.

THE year 1972 was the hundredth anniversary of the publication of the Boltzmann equation—apparently an auspicious year for the publication of a book on the kinetic theory of gases as based on this famous equation. This theory gives a complete prescription for calculating transport properties of dilute gases from knowledge of the intermolecular forces. As such, it is one of the most elegant and highly-developed physical theories available. But any book published in 1972 must face a severe test—comparison with two well known monographs, *The Mathematical Theory of Non-Uniform Gases* by Chapman and Cowling, now in its third edition (1970), and *Molecular Theory of Gases and Liquids* by Hirschfelder, Curtiss and Bird, in its second printing (1964). These are rightly regarded as classics, and have set a standard by which all other books in the field are measured. Ferziger and Kaper have attempted the difficult task of combining the best features of both of these works in a textbook for graduate study. Let me quote their own words for the reasons for the attempt. Chapman and Cowling ". . . is rather difficult as a text and does not emphasise the calculation of transport properties sufficiently. . . . Hirschfelder, Curtiss and Bird, while very good in discussing the calculation of transport properties, compresses the theory far more than desirable".

In my opinion the authors have succeeded admirably in their aim. There is no doubt that a real need exists for such a book, as evidenced by a prevailing opinion to the effect that ". . . reading Chapman and Cowling is like chewing on broken glass; reading Hirschfelder, Curtiss, and Bird is like punching a feather pillow". I wish a book like this had existed when I first started to try to understand kinetic theory.

This book covers much the same ground as Chapman and Cowling, but with more emphasis on intermolecular forces and the computation of transport coefficients. There are also excursions beyond the range of the Boltzmann equation to include modern work on dense gas theory and some discussion of rarefied gas dynamics. A book like this cannot hope to be complete, and in some areas will become out of date very quickly. One can see some signs of this already happening, but I do not feel that this detracts appreciably from the book's value. The discussion of fundamentals and mathematical developments is so well done as to be of almost permanent value, and the treatments of the more rapidly changing areas will enable the reader to proceed for himself into the research literature.

Finally, it is interesting to note that the book developed from a course intended for aeronautical, mechanical and chemical engineers. It should be of equal value to physicists and chemists, and even to biologists interested in phenomena describable by Boltzmann-type equations. I predict that it will be a standard text for some time to come. E. A. MASON

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Applications, giving details of academic record and curriculum vitae should be posted to Professor D. G. Roux, Department of Chemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, South Africa. (31)

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Applications, with the names of two referees, should be sent to Dr. D. M. Finlayson, Department of Physics, The University, St. Andrews, Fife, not later than January 15, 1974. (22)

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Miss A Waring
Personnel Officer
Imperial Chemical Industries Ltd
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For further details and an application form (to be returned by January 25, 1974) write to Civil Service Commission, Alencon Link, Basingstoke, Hants; or telephone Basingstoke 29222, ext. 500, or London 01-839 1992 (24-hour answering service), quoting S/8495. (13)

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Applications (six copies) should be lodged, not later than February 1, 1974, with the undersigned, from whom further particulars may be obtained.

In reply please quote Ref. No. 3394M.

ROBT. T. HUTCHESON,
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(39)

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Application forms may be obtained from the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. (63)

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UNIVERSITY OF OXFORD. LECTURESHIP IN ORGANIC CHEMISTRY. Applications are invited for a University Lectureship in the Dyson Perrins Laboratory, to be held in conjunction with the Fellowship at Wadham College advertised below. Salary in range £1,929 to £4,863 (depending upon age), plus F.S.S.U.—Further particulars from the Administrator, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, to whom 6 copies of typed applications (only 1 from overseas candidates) should be sent no later than February 4.

WADHAM COLLEGE. OFFICIAL FELLOWSHIP IN ORGANIC CHEMISTRY. The College proposes to elect an Official Fellow in Organic Chemistry from October 1, 1974. Both men and women are eligible. The post is to be held in conjunction with the University Lectureship in the Dyson Perrins Laboratory advertised above.—Applications, giving personal details and a brief statement of academic record, should be sent not later than February 4, 1974, to the Warden, from whom further particulars, including the stipend and teaching duties, may be obtained. (6)

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DEPARTMENT OF AGRONOMY—(a) PROFESSOR OF AGRONOMY. (b) AGRONOMIST/BREEDER (Cereals). (c) AGRONOMIST (Grain Legumes). (d) AGRONOMIST (Fibres). Candidates for (a) should have a good honours degree in agriculture or a related science. A higher degree or postgraduate training is necessary. Considerable experience in agronomic research, dealing with the principles and practices of crop production and field management, is essential. Administrative ability with academic experience in a university department is required to provide leadership in the teaching and research programme. Appointee will guide the educational and research programmes in all aspects of the growth and development of crops in the areas of agronomy and physiology in a department with an establishment of 24 senior staff. In addition to traditional agronomic investigations, he will organise multidisciplinary studies on the production aspect of farming systems. He will be responsible for the agronomic operation and organisation of experimental trials in the six States, the maintenance of a central repository of records and for statistical services in the Institute. He will be expected to contribute to the teaching programme and its development in the area of his specialisation to students of the Faculty. He will have the opportunity to develop an M.Sc. degree programme for majors in agronomy.

Posts (b), (c) and (d) will be offered at Research Fellow or Assistant Research Fellow level, and candidates should have a good honours degree in agronomy or breeding. A higher degree or evidence of postgraduate experience is necessary for appointment at Research Fellow level. Appointee for (b) must have some knowledge of cereals (primarily maize or sorghum breeding techniques) and be able to conduct field trials leading to improved cereal production. For (c), appointee must be able to conduct agronomic and nutrient research leading to improved production of grain legumes. For (d), appointee will be expected to conduct research with vegetable fibre crops (kenaf, jute, etc.) leading to improved production of fibre. He will also be expected to pay attention to the prospects of paper production from the crops.

DEPARTMENT OF PLANT SCIENCE—(a) RESEARCH FELLOW IN COTTON BREEDING and (b) RESEARCH FELLOW IN HORTICULTURE in the Cotton Breeding and Botany Sections. Candidates for (a) should hold a good first degree in agricultural botany, botany or agriculture and have research experience in plant breeding. Field experience in the tropics would be very desirable. Appointee will initially be stationed at the headquarters of the Institute at Samaru, Zaria, where he will join a team of plant breeders which is charged with the responsibility for the improvement in yield, disease resistance and quality of the cotton crop in the Northern States of Nigeria. Candidates for (b) should hold a good first degree in agricultural botany, botany or horticulture and have research experience, preferably in plant breeding. Field experience in the tropics would be very desirable. Appointee will initially be stationed at the headquarters of the Institute at Samaru, Zaria, where he will be responsible for the onion breeding programme and for research on other crops, including peppers and local vegetables.

Salary scales: Professor N6,600 per annum. Research Fellow N2,760 to N4,830 per annum. Assistant Research Fellow N2,140 to N2,660 per annum. (£1 sterling=N1.59.) The British Government may supplement the salaries of Professors by £1,350 per annum (sterling) for married appointees, or £800 per annum (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. The supplementation is unlikely to be applied to appointments at Research Fellow or Assistant Research Fellow level. Family passages; various allowances; superannuation scheme; biennial overseas leave.

Detailed applications (two copies), including a curriculum vitae and naming three referees, should be sent by airmail, not later than January 22, 1974, to the **Registrar, Ahmadu Bello University, Zaria, Nigeria.** Applicants resident in U.K. should also send one copy to **Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT.** Further particulars may be obtained from either address. (2070)

The **MAX-PLANCK-GESELLSCHAFT** zur Förderung der Wissenschaften e.V. provides in the **Friedrich-Miescher-Laboratorium für biologische Arbeitsgruppen** in Tübingen opportunities for

YOUNG SCIENTISTS

to lead research groups of their own and to carry out independent research programmes for a fixed period of time.

Three out of four of these positions will have to be filled in; one will become available at once and the two others in the fall of 1974.

Applicants should already have done some recognised research in their own particular field, preferably in the areas of **neurobiology** and **cellular differentiation.**

Appointments will be limited to five years. Salaries will fall within the range AH 3 of the Niedersächsisches Besoldungsgesetz (about 45,000 DM per annum).

Each research group will have available approximately 150 square metres of floor space, plus adequate means for scientific and technical supporting staff, basic equipment and operating expenses.

Applications should be submitted before February 15, 1974, to the

Max-Planck-Gesellschaft
zur Förderung der Wissenschaften e.V.
–Generalverwaltung–III a 1–
8000 München 1
Postfach 647, Germany.

(32)

Glaxo

Graduates? HNC?

Make a career in Fermentation Research

Our Fermentation Research Department is located in pleasant parkland surroundings at Sefton Park near Stoke Poges in Buckinghamshire.

We are looking for people to be responsible in our Pilot plant, on a 6 days on—2 day off shift basis, for planning the work of a team of process workers, and supervising plant operations, including the expanding use of modern instrumentation.

Ideally you should be qualified to degree or HNC level in a biological science and be familiar with Pilot scale fermenters, or have a good background in microbiology and aseptic techniques. However we would be prepared to consider those qualified to ONC standard, who have suitable experience.

In addition to an attractive salary, the company, which is a multi-million international group, offers good conditions of employment, including bonus and pension scheme. Assistance with relocation expenses will be given where appropriate.

Please write giving brief career details to: Personnel Officer, REN (Research), Glaxo Laboratories Limited, Greenford, Middlesex.

(47)

Wellcome

Senior Technician

Electron Microscopy

We are looking for a Senior Technician to take charge of the general day-to-day operation of The Electron Microscope Suite. This section is equipped with two electron microscopes, freeze-etching plant, ultramicrotomes and other necessary ancilliary equipment, and provides a service to scientists of all disciplines.

The successful candidate will be between 25 and 35, with at least 5 years' experience of electron microscopy in the biological field, and be capable of working in a multi-discipline environment.

This is an important post and offers excellent conditions of employment and salary commensurate with age, qualifications and experience.

Our Laboratories are situated in pleasant parkland surroundings within easy reach of Beckenham, Bromley and Croydon.

Please write quoting reference U.430 and giving brief details of qualifications and experience to The Senior Personnel Officer, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS.

1971

(46)

PROFESSOR AND CHAIRMAN
DEPARTMENT OF NUTRITION
UNIVERSITY OF GUELPH

An individual with a scholarly reputation and considerable experience in the biochemical aspects of nutrition is sought for this position. In addition to administrative duties, the Chairman is expected to participate in the teaching activities of the department and to establish his own research programmes. The initial appointment will be for five years, with five year extensions subject to the approval of a review committee. The salary is commensurate with qualifications and experience.

The department is located in the College of Biological Science and currently consists of ten full-time faculty members. It has a strong graduate programme at the M.Sc. and Ph.D. levels and is actively involved in undergraduate teaching. A new B.Sc. degree programme in honours nutrition and biochemistry is being introduced in spring 1974.

Applications, curriculum vitae, and the names of three referees, should be sent to Professor K. Ronald, Dean, College of Biological Science, University of Guelph, Guelph, Ontario N1G 2W1. (28)

CITY OF LONDON POLYTECHNIC
DEPARTMENT OF PHYSICS
LECTURER (GRADE II)

A vacancy exists for a Lecturer (Grade II) who will be required to undertake teaching duties at all academic levels, and in particular to make a substantial contribution to the teaching of the M.Sc. course in optical aspects of molecular and polymer physics. Relevant industrial experience in polymer physics would be an advantage. It is expected that the successful candidate would be appointed from April 1, 1974, but a later appointment is not precluded.

Salary: Lecturer Grade II, £2,515 to £3,243 plus £118 London Allowance.

Further particulars and application form (which should be returned by January 18, 1974) may be obtained from the Assistant Secretary, City of London Polytechnic, 117-119 Houndsditch, London, E.C.3. (49)

UNIVERSITY OF SURREY
VICE-CHANCELLOR

The Vice-Chancellor, Dr. D. M. A. Leggett, has notified the University of his intention to retire on September 30, 1975. The Joint Committee of Council and Senate charged with finding a successor have considered the nature of the appointment and the likely background of the person they hope to find. A note of this information is available.

Persons interested in this appointment are invited to write to the undersigned for a copy. The strictest confidence will be maintained.

P. J. Timms,
Clerk of the University Council,
University of Surrey, Guildford.

(45)

UNIVERSITY OF ADELAIDE

Applications are invited for the following appointment:

TEACHING FELLOW IN ELECTRICAL ENGINEERING with interests in communication systems, signal processing, speech analysis, system assessment, power system control, microwave engineering. Interaction with industry is encouraged. (1.2.74.)

Salary scale: Teaching Fellow $A7,270 by $A286 (3) by $A285 (2) to $A8,698; a non-superannuated and non-tenured appointment.

Further particulars about this post and the conditions of appointment and other information sought will be supplied on request to the Registrar of the University, or to the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications should be sent in duplicate and giving the information listed in the Statement that will be supplied to the Registrar, The University of Adelaide, North Terrace, Adelaide, South Australia 5001.

(54)

UNIVERSITY OF LONDON
THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
INSTITUTE OF NUCLEAR MEDICINE

Applications are invited for the post of RESEARCH ASSISTANT to join a group studying hormone target-cell-receptor interactions. The successful applicant will work mainly with gonadotrophins. Experience of cell fractionation procedures is desirable but not essential. Salary, according to qualifications, age and experience, within the range £1,602 to £2,223 per annum plus £162 London Allowance and superannuation under F.S.S.U.

Applications from suitably qualified graduates, including a curriculum vitae and the names and addresses of two referees, to Professor E. S. Williams (N), Institute of Nuclear Medicine, The Middlesex Hospital Medical School, Nassau Street, London W1N 7RL, by January 31, 1974. (17)

AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF PHYSICAL SCIENCES

PROFESSORIAL FELLOW IN ASTRONOMY

Applications are invited for the post of Professorial Fellow in the Department of Astronomy, which operates the Mount Stromlo and Siding Spring Observatory (Head: Professor O. J. Eggen).

At present the main fields of investigation are: photometry and spectroscopy of stars in advanced evolutionary phases, the study of stellar pulsations, interstellar and intrinsic polarization, optical and radio studies of galaxies, the composition of stellar atmospheres, and the study of globular clusters. Theoretical work at present being carried out includes studies of model stellar atmospheres, of the interior structure of stars in advanced evolutionary phases, and of galactic dynamics. Siding Spring Mountain has been selected as the location for the 150-inch Anglo-Australian telescope. Along with other Australian astronomers, the successful applicant may apply for time on this telescope.

In addition to participating in the normal research activities of the Department, the appointee will have special responsibilities for the supervision of scholars and participation, through an instrument committee, of all development (especially electronic) and construction of observatory instruments.

The salary of a Professorial Fellow is $A17,648 per annum. Superannuation is on the F.S.S.U. pattern with supplementary benefits. Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Further information may be obtained from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close on **18 February, 1974.** (53)

DALHOUSIE UNIVERSITY

DEPARTMENT OF MICROBIOLOGY

Applications are invited for a Faculty position at the level of LECTURER/ASSISTANT PROFESSOR. Applicants should have an M.D. or Ph.D. with research experience and a major interest in medical microbiology. Salary according to qualifications and experience. Appointment effective July 1, 1974.

Applications to Dr. K. B. Easterbrook, Department of Microbiology, Dalhousie University, Halifax, N.S., Canada, with curriculum vitae and names of three referees. (30)

ICI

Imperial
Chemical
Industries
Limited

Pharmaceuticals Division

Experimental Officer Parasitology

There is a vacancy for a graduate Biochemist to work on the development of biochemical tests, evaluation of potential enzyme inhibitors in fluke and worms, and the exploration of host-parasite metabolic differences as basis for design of drugs. An interest in the application of biochemical knowledge and techniques to biological problems is essential. Practical experience with biochemical techniques is desirable.

The Pharmaceuticals Division is very attractively situated in rural North Cheshire, within easy reach of main road and rail routes. There is a wide range of country and town housing within 20 minutes' drive to cater for most tastes. Conditions of service, career opportunities, and assistance given to married men in moving home are designed to attract and retain staff of high calibre.

Applications in writing, giving details of age, qualifications and experience, should be addressed to:

M F Losse, Personnel Officer
Imperial Chemical Industries Ltd
Pharmaceuticals Division
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4TG

(57)

Organon Research

Organon Research & Development Laboratories are engaged in a broad programme of research, aimed at the development of new drugs.

Organon R & D Laboratories are an international multidisciplinary scientific organization of about 650 collaborators of which 500 are located in Oss, The Netherlands. It maintains close contacts with scientific institutions all over the world.

In the Pharmacological R & D Laboratories, a vacancy has become available for a

Research Oriented Physician

or

Medically Oriented Biologist

The successful candidate will in due course head one of our CNS teams concerned with the study of new psychopharmaca by means of electrophysiological, neuropharmacological or animal-psychological techniques. Development of new methods for the study of mechanisms of action of drugs by means of animal experiments is strongly emphasized.

He will be a creative researcher of 30 to 40 years of age and be familiar with one or more of above mentioned techniques in addition to having a broad pharmacological knowledge. Remuneration will be commensurate with the responsibilities of this important position and the experience of the candidate.

Applications should be directed to the Personnel Department, Organon International BV, P.O. Box 20, Oss (The Netherlands), under No. C. 1464.

Organon

Organon International BV
subsidiary of Akzo Pharma

(11)

MURDOCH UNIVERSITY

Perth, Western Australia

SENIOR LECTURESHIPS AND LECTURESHIPS

Murdoch University, Western Australia's second university, will open in 1975 with about 500 undergraduate students. The university is committed to taking a fresh approach to higher education, with the goal of responding directly to the conditions and needs of the late twentieth century. This will be reflected in admission policies, organisational structures, programmes of study, philosophies of teaching and recruitment of staff. Applications are now invited for up to 30 posts as Lecturer and Senior Lecturer, to take up appointment towards the end of 1974 or early 1975.

Appointments have been made to Chairs in Biology, Chemistry, Education, Environmental Studies, History, Literature, Mathematics, Physics, Social Science, and Veterinary Studies, and further professorial appointments will shortly be made in Animal Biology, Applied Veterinary Medicine, Economics, Psychology, and Veterinary Biology. The university will be organised into six Schools of Study, namely, Schools of Education, Environmental and Life Sciences, Human Communication, Mathematical and Physical Sciences, Social Enquiry, and Veterinary Studies. The undergraduate curriculum will be divided into Part 1 and Part 2 Courses of Study, the Part 1 Course being administered by a university-wide board. A good deal of choice by students will be encouraged and broad contextual courses will be offered, as well as introductory courses in the disciplines forming the foundations of Part 2 programmes of study. Part 2 programmes will be administered largely by multi-disciplinary staff committees. The programmes which it is hoped to offer from 1976 are:

Applied Micro-biology (Industrial)
Biology
Chemistry
Chinese Studies
Communication
Economics
Education
Environmental Science (especially synecology, atmosphere science and waste disposal)
History (especially modern social history)
Human Development (biological, psychological and social)
Indonesian Irenology (the study of conflict and its resolution)
Mathematics
Mineral Science
Physics
Population and World Resources
Veterinary Biology
World Literature

Applications are invited from appropriately qualified persons in all disciplines to participate in introductory teaching at Part 1 level in 1975, and to contribute to the planning and teaching of one or more of the Part 2 programmes from 1976.

The university has decided from the outset to offer external tuition towards degrees. This will be the responsibility of the staff of the schools of study, and the external studies programme as a whole will be coordinated by a Director of External Studies to be appointed in mid-1974.

Along with details of their qualifications, experience, teaching and research interests and publications, applicants should include **in their applications** a note about the programme to which they believe they would be competent to contribute, together with a brief statement of their special interests in these programme areas.

Salaries: Senior Lecturer $A12,268 to $A14,308 per annum; Lecturer $A8,698 to $A11,984 per annum.

Further information about Murdoch University and these appointments, including details of superannuation, travel and removal expenses, housing assistance, study leave and the conditions of employment is available from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, or from the Secretary, Murdoch University, Murdoch 6153, Western Australia.

Applications will close on **February 18, 1974.** (52)

SOUTH-EASTERN REGIONAL HOSPITAL BOARD, SCOTLAND

Applications are invited for the new post of **BASIC-GRADE BIOCHEMIST** in the Regional Hormone Laboratory shortly to be established at 2 Forrest Road, Edinburgh, under the direction of Dr E. H. D. Cameron.

The responsibilities of the post will involve the setting up and running of steroid hormone radioimmunoassays as a service for the region, and the successful candidate will be expected to assist in research and development of new techniques.

The Regional Hormone Laboratory is a sub-department of the Department of Clinical Chemistry, Royal Infirmary, and there will be opportunities for staff to rotate for training purposes through this and other clinical chemistry departments in this region if desired.

Further particulars on application.

Applications, which should be typewritten, giving details of age, qualifications and previous experience, together with the names and addresses of two referees, should be sent to the Secretary, 11 Drumsheugh Gardens, Edinburgh EH3 7QQ, by January 12, 1974. (43)

UNIVERSITY OF LEICESTER

READER OR SENIOR LECTURER IN GENETICS

Applications are invited for the post of READER or SENIOR LECTURER in the Department of Genetics. Applicants should have experience in teaching medical students and an interest in human or medical genetics. The person appointed will be responsible for the development of these aspects of genetics in the department consequent upon the foundation of the new medical school, and will be expected to take part in the promotion of developmental medicine in the teaching hospitals. A medical qualification is therefore desirable but not essential.

Level of appointment and initial salary, dependent on qualifications and experience, within the scale £4,368 to £5,496 a year, with F.S.S.U. membership.

Further particulars from the Registrar, to whom applications should be sent by January 28. (74)

Kings
COLLEGE HOSPITAL GROUP

RESEARCH ASSISTANT

A vacancy exists for an Assistant to a group working on cancer research, experimental radiotherapy of tumours in animals, and growth control mechanisms. Salary according to experience.

Applications to the Principal Technician, Division of Pathology, King's College Hospital, Denmark Hill, London SE5. (20)

KENNEDY INSTITUTE

DIVISION OF EXPERIMENTAL PATHOLOGY

CHIEF TECHNICIAN

A Chief Technician is required to organize and administer the technical services of this active research group concerned with the structure and function of lymphoid tissues combining morphological and biochemical studies, with particular reference to nucleic acids. Appropriate qualifications for the seniority of the post would be required, together with background experience in relevant techniques which include histology, cytochemistry, autoradiography, immunology, tissue and organ culture.

Salary within Whitley Council range for Chief Technicians (£2,589 to £3,132 per annum, plus London Weighting).

Written applications, naming two referees, to: The Laboratory Superintendent, The Kennedy Institute, Bute Gardens, Hammersmith, London W6 7DW. (27)

DEPARTMENT OF BIOLOGY
HARVARD UNIVERSITY

For the forthcoming academic year, this department expects to make at least two appointments at the ASSISTANT PROFESSOR level. Fields of interest include plant biology, genetics, immunology and developmental biology. The university is an equal opportunity employer; women and members of minority groups are specifically encouraged to apply.

Please have three professional persons write us concerning you, and send your résumé, together with your most significant publications prior to February 15, 1974, to Chairman, Department of Biology, 16 Divinity Avenue, Cambridge, Massachusetts 02138. (5)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)

Keppel Street, Gower Street, London W.C.1

Clinical Nutrition and Metabolism Unit

READVERTISEMENT

TECHNICIAN, graduate or H.N.C., required immediately to join an M.R.C. supported group studying problems of biochemical adaptation in relation to obesity and surgical trauma. The group is situated at the Hospital for Tropical Diseases, St. Pancras, N.W.1. Applicants should have a good biochemical background and laboratory experience. Salary, according to qualifications and experience, within the scale £1,440 to £1,845 plus £126 London Weighting.

Applications, consisting of full curriculum vitae and naming two referees, should be sent to the Secretary (A1) at the School. (16)

COLÁISTE NA hOLLSCOILE, GAILLIMH

(UNIVERSITY COLLEGE, GALWAY)

University Senior Technician/ Electronics Technician

with an interest in telecommunications required in a University Department. City and Guilds qualifications or equivalent necessary.

Apply to:

Professor of Physiology,
University College,
Galway. (64)

UNIVERSITY OF CALGARY

Applications are invited from students wishing to conduct GRADUATE STUDIES IN BIOLOGICAL SCIENCES for the Ph.D. or M.Sc. degree. Successful applicants may compete for Teaching and Research Assistantships which carry a stipend, remission of fees and a travel allowance.

For further information write to: The Secretary for Graduate Studies, Department of Biology, University of Calgary, Calgary T2N 1N4, Alberta, Canada. (36)

CORNELL UNIVERSITY

SECTION OF NEUROBIOLOGY AND BEHAVIOUR

Cornell University is seeking a ZOOLOGIST (Ethologist) specializing in animal social behaviour, with an emphasis on the field approach. Teaching and research duties. Assistant Professor rank, salary negotiable. Send credentials or inquiries to:

Professor William T. Keeton, Chairman,
Section of Neurobiology and Behavior,
Cornell University,
Ithaca, New York 14850.

Cornell University is a non-discriminatory, equal opportunity employer. (51)

M.R.C. MINERAL METABOLISM UNIT

THE GENERAL INFIRMARY LEEDS

GRADUATE in one of the biological sciences required to work on the biochemistry and physiology of vitamin D and other aspects of calcium metabolism. Salary according to age, experience and qualifications.

Please apply with details of qualifications to the Director. (24)

UNIVERSITÄT ZU KÖLN GERMANY

INSTITUT FÜR GENETIK

E. COLI GENETICIST

A post-doctoral research position for work in association with Professor B. Müller-Hill will be available immediately. The appointment will be for two to three years. The salary will be on the scale of ca. 27,000,— DM per annum.

Applications, with curriculum vitae and the names of two referees, should be sent to Professor B. Müller-Hill, Institut für Genetik der Universität zu Köln, Köln 41, Weyertal 121, West Germany. (25)

AQUATIC ECOLOGIST

Appointment available at Rosenstiel School of Marine and Atmospheric Science, University of Miami, for an Aquatic Ecologist interested in quantitative population or community ecology, with particular interest in food chain dynamics and community structure. Responsibilities will include teaching and the designing and co-ordination of field research. Level of appointment would be commensurate with qualifications.

Inquiries should be submitted to:

James H. Carpenter,
Chairman, Search Committee,
R.S.M.A.S., University of Miami,
10 Rickenbacker Causeway,
Miami, Florida 33149.

The University of Miami is an equal opportunity/affirmative action employer. (26)

UNIVERSITY HOSPITAL OF WALES

HEATH PARK, CARDIFF

Applications are invited from suitably qualified people for the post of MEDICAL LABORATORY TECHNICIAN in the Department of Haematology at Cardiff Royal Infirmary. Duties will include assistance with routine microbiological assays and with the current investigation into the automation of these techniques.

APPLICATIONS: Application forms available from the Personnel Department. (18)

UNIVERSITY OF CALGARY, GRADUATE Teaching and Research Assistantships are available for students proceeding to the Ph.D. or M.Sc. degrees in all branches of Chemistry and Biochemistry. The Assistantships are valued from $4,100 to $5,000 per annum and carry remission of programme fees and travel allowance.

For further information write to: Department of Chemistry, Calgary, Alberta T2N 1N4, Canada. Please indicate field of interest. (X35)

ROYAL POSTGRADUATE MEDICAL SCHOOL

GRADUATE SCIENTIFIC OFFICER/TECHNICIAN required in Immunoassay Laboratory of the Endocrine Unit. Candidates should possess good biochemical and isotope qualifications and laboratory experience, preferably in a similar post. Salary, according to qualifications and experience, in range £1,600 to £2,300.

Applications to the Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, DuCane Road, W12 0HS, quoting reference 4/111/N. (70)

Overseas Appointments

BELIZE

Tobacco Officer:

To initiate production amongst small holders, and provide advice to growers; to train local staff and assist with trials. Applicants should have experience with Virginia and Burley tobacco, and be experienced in the prescribed duties of the post.

Salary in scale £3,000–£4,500 p.a. plus a tax free overseas allowance in scale £435–£1,145 p.a. Appointment for 2 years.

BRAZIL

Professor of Forestry:

At the Federal University to lecture to post-graduate students on forest management and land use; to supervise research projects. Applicants should hold relevant post-graduate qualifications and be experienced in the prescribed duties of the post; knowledge of forest ecology an advantage but not essential. Appointment of 2 years.

Salary in scale £4,500–£6,400 p.a. plus a tax free overseas allowance in scale £650–£1,390 p.a.

ST VINCENT

Agricultural Engineering Technician

To undertake technical back-up to arrowroot harvesting; advise on introduction of field machinery; advise on irrigation and soil conservation; and conduct relevant training courses. Applicants aged 25–40 should be professionally qualified in Agricultural Engineering Technology and have practical experience in the prescribed duties of the post. Appointment for 2 years.

Salary in scale £2,400–£4,000 p.a. plus a tax free overseas allowance in scale £750–£1,435 p.a.

Other benefits include free family passages, paid leave, children's education allowances and free accommodation and medical attention. Applicants should normally be citizens of, and permanently resident in, the United Kingdom. All emoluments paid by British Government.

For full details together with an application form and booklet about the country concerned please apply giving details of age, qualifications and experience to:—

Appointments Officer,
Foreign & Commonwealth Office

Overseas Development
Administration

Room 301/N, Eland House, Stag Place,
London, SW1E 5DH

(62)

COLLEGE OF PHARMACY
FACULTY OF HEALTH PROFESSIONS
DALHOUSIE UNIVERSITY, CANADA

ASSISTANT PROFESSOR IN PHARMACEUTICS

Ph.D. in Pharmaceutics with major in Physical Pharmacy required for July 1, 1974, for a new faculty position. Teaching and research experience desirable. Research actively encouraged. Salary according to experience and qualifications.

Applications in the form of a letter accompanied by a curriculum vitae and the names and addresses of three referees should be sent to Dr R. S. Tonks, Director, College of Pharmacy, Dalhousie University, Halifax, Nova Scotia.

(50)

STATISTICAL TECHNIQUES

The Company is expanding the development and production of clinical assay kits utilising radioisotopes. New techniques and approaches are constantly being examined for the quality control of these products and we need a statistician to advise and collaborate with scientists and technologists in these areas.

The ideal person for this post would probably be aged 26-32 and have an honours degree in statistics. Experience in a scientific or bio-medical environment is desirable though not essential. Applications are also invited from suitably qualified but perhaps less experienced people who feel ready for the challenge of being the sole professional statistician in a multi-disciplinary team.

Good computing facilities are available, and it is intended that, whilst clinical assay will be the prime area of involvement, the person appointed will increasingly bring his professional approach to bear on other aspects of the Company's work.

A starting salary of up to £3500 is envisaged (it could be higher for the exceptional person) on a progressive scale offering prospects to £4900 and beyond.

Please write to or ring Dr. T. E. Forster for an application form and further particulars. (Telephone Little Chalfont 4444).

The Radiochemical Centre

Amersham Bucks

(19)

SOUTH-EASTERN REGIONAL HOSPITAL BOARD, SCOTLAND

Applications are invited for the new post of **SENIOR GRADE BIOCHEMIST** in the Regional Hormone Laboratory shortly to be established at 2 Forrest Road, Edinburgh, under the direction of Dr E. H. D. Cameron. The responsibilities of the post will involve supervision of service protein hormone assays, and every opportunity will be afforded to the successful candidate to undertake research and development of new techniques, and to cooperate in clinical research programmes.

The Regional Hormone Laboratory is a sub-department of the Department of Clinical Chemistry, Royal Infirmary, and there will be opportunities for staff to rotate for training purposes through this and other clinical chemistry departments in this region if desired.

Further particulars on application.

Applications, which should be typewritten, giving details of age, qualifications and previous experience, together with the names and addresses of two referees, should be sent to the Secretary, 11 Drumsheugh Gardens, Edinburgh EH3 7QQ, by January 12, 1974. (42)

INSTITUTE OF CONNECTED EDUCATION

offer prizes for research in pure general science. That is, research in interdisciplinary sciences, or if and how all sciences are interconnected. Main object of the Institute is to create and sponsor the acceptance of a course of this nature in schools, colleges and institutes.

For further information please apply in writing to: **The Secretary, Institute of Connected Education, 28a Cadogan Place, London SW1X 9RX.**

(40)

PONTEFRACT, CASTLEFORD AND GOOLE HOSPITAL MANAGEMENT COMMITTEE

BIOCHEMIST BASIC GRADE

required—Pontefract General Infirmary. Well-equipped department in developing general hospital. New maternity unit recently commissioned. Training will be given in all branches of clinical biochemistry, and specialisation encouraged. Starting salary according to qualifications and relevant postgraduate study or research. On scale £1,380 to £2,122 per annum, during probationary period of at least two years, thereafter subject to a certificate of proficiency to a maximum of £2,517.

For further information and arrangements to visit contact Mr R. Lockwood, Principal Biochemist, tel. Pontefract 72361, ext. 238. Applications, giving full details and naming two referees, to Group Secretary, Churchill House, 29 Mill Hill, Pontefract, by January 14, 1974. (68)

UNIVERSITY COLLEGE LONDON CHEMISTRY DEPARTMENT

TECHNICIAN (GRADUATE) to assist in research involving wide line (other-nucleus) N.M.R. Interest in electronics essential. Post is financed by S.R.C. and has not less than 2 years to run in the first instance. Salary range £1,650 to £1,920, plus £175 London Allowance.

Applications to Mr. F. Widdas (N), Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ. (15)

UNIVERSITY OF MANCHESTER

RESEARCH ASSISTANT IN THE DEPARTMENT OF CHILD HEALTH

Applications invited for this post from graduates, preferably with post-doctoral experience, to work on tissue culture of endocrine pancreas. Experience in cell biology, endocrinology or biochemistry an advantage. Appointment is for two years from March 1, 1974. Salary range £2,718 to £3,048 per annum. F.S.S.U.

Particulars and application forms (returnable by January 18, 1974) from Dr. R. D. G. Milner, St. Mary's Hospital, Manchester M13 0JH. (60)

AGRICULTURAL RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE

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Superannuation: Optional Contributory scheme.

Application form and further details from the Secretary, Food Research Institute, Colney Lane, Norwich NOR 7OF, quoting Ref. 73/26. (69)

MARINE SCIENCE TEAM

Geological Oceanographer-Geophysicist, Ph.D., 20 years' experience in continental shelf exploration, research and teaching, with numerous publications and some patents pending, and **Biological Oceanographer,** M.Sc., specialist in marine microbiology and pollution, presently teaching and operating own research vessel in the eastern Mediterranean, are interested in becoming associated with an aggressive, developing research, academic or industrial organisation, preferably Europe or Mediterranean area.—Write Box 2079, Nature, T. G. Scott & Son Ltd., Clement's Inn, London WC2A 2ED. (7)

UNIVERSITY OF READING, LECTURESHIP IN GEOLOGY. Applications are invited for a Lectureship in Geology. Candidates will be expected to teach in sedimentology and closely related areas, and to have a major research interest in the chemical and/or biological aspects of this field. The person appointed should take up duties on October 1, 1974.—Further information may be obtained from the Registrar (Room 208, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, and applications should reach him not later than **February 4, 1974.**

(33)

Manager

(Precious Metal Catalyst Research)

Johnson Matthey, as the world's major refiner and fabricator of the platinum metals, has extensive interests in the development of catalysts and catalytic processes for use in pollution control and in the chemical and pharmaceutical industries.

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The post will be particularly attractive to those aged between 30-45 with previous industrial experience in catalyst research and development, or who have a proven record of academic achievement and are now interested in applying their experience in an industrial field.

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Applications giving brief but comprehensive career details should be addressed to: **Director of Research (ref. SJTH), Johnson, Matthey & Co., Limited, 78 Hatton Garden, London, EC1P 1AE.** (78)

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INSTITUTE OF CANCER RESEARCH: Department of Physics. PHYSICIST or ELECTRICAL ENGINEER required at Belmont, Sutton, Surrey, to assist in the development of novel techniques in (a) X-ray image formation for medical diagnosis and (b) studies of the action of ionising radiation on biological materials using short pulse irradiation. some research or industrial experience with ionising radiation would be an advantage but not essential. Salary on M.R.C. scales Grade II, depending on qualifications and experience.—Apply in duplicate, giving curriculum vitae and quoting two referees, to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting Ref. No. 300/G/65. (61)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited from suitably qualified candidates for the above post.

The person appointed will be engaged on a project supported by the Medical Research Council involving the study of steroid hormones secreted by the ovary under *in vitro* superfusion conditions.

The appointment will initially be for 18 months, and the post will be available from April 1, 1974. Salary in the range £1,929 to £2,223.

Applications, with a curriculum vitae, together with the names of two referees, and quoting Ref. R35/73, should be sent to Dr J. Watson, Department of Biochemistry, University of Strathclyde, 204 George Street, Glasgow G1 1XW. (38)

UNIVERSITY OF READING, LECTURESHIP IN GEOGRAPHY (readvertisement). Applications are invited for a Lectureship in Geography. Applicants should be competent in hydrology and/or microclimatology. The person appointed should take up duties on the earliest date which can be arranged.—Further information may be obtained from the Registrar (Room 208, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, and applications should reach him not later than **January 31, 1974.** (34)

GRANTS & SCHOLARSHIPS

THE ROYAL SOCIETY
THE RUTHERFORD SCHOLARSHIP

Applications are invited from graduates of a university within the British Commonwealth who are under 26 years of age, or if holding the degree of Ph.D. under 28 years of age on May 1, 1974, for the RUTHERFORD SCHOLARSHIP. This will be awarded for experimental research in any branch of the natural sciences, but if there are candidates of similar merit preference will be given to experimental physics. The scholarship will be tenable for three years in some part of the British Commonwealth other than that in which the applicant graduated. It will not normally be awarded to a graduate who has already held a senior research award.

The value of the scholarship will be adjusted to meet the circumstances of the appointment, but if held in the United Kingdom it will be between £950 and £1,200 per annum (or up to £1,600 per annum for post-doctoral candidates). Additional allowances will be granted for travel, university fees, etc. The appointment will date from October 1, 1974, or such other date as may be arranged.

The appointment will be made by the President and Council of the Royal Society. Application from university graduates outside the United Kingdom should be made through their universities to the Royal Commission for the Exhibition of 1851 on forms of application for the 1851 Exhibition Science Research Scholarship. Application from university graduates in the United Kingdom should be made before February 15, 1974, on forms obtainable from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG. (29)

FELLOWSHIPS AND STUDENTSHIPS

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Applications, giving full bibliographical details and the names of two referees who may be contacted should be sent to Professor H. Ross. (41)

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF CHEMICAL ENGINEERING AND FUEL TECHNOLOGY

POST-DOCTORAL FELLOWSHIP AND RESEARCH ASSISTANT

Applications are invited for a Post-Doctoral Fellowship in the field of Air Pollution from Aircraft. The research work is supported by a grant from the United States National Aeronautics and Space Administration and will be carried out as part of a joint research project with the Department of Mechanical Engineering at the Massachusetts Institute of Technology in Boston. Experiments will be carried out at Sheffield University on the combustion efficiency and pollution characteristics of swirler modules used in the N.A.S.A. gas turbine combustion chambers. Velocities will be measured in the flames by a laser anemometer, and temperatures and gas concentrations will also be measured in the flames. Provision is made in the budget to allow visits to M.I.T. and N.A.S.A. Research Centres in the United States. Initial salaries are according to experience, up to £3,048 for the Post-Doctoral Fellow and up to £2,058 for the Research Assistant. The Research Assistant may be able to register for a Ph.D., in which case a limited salary will be imposed.

Applications, giving the names of two referees, should be sent to Dr N. A. Chigler, Reader in Chemical Engineering and Fuel Technology, The University, Sheffield S1 3JD. Please quote Ref. R12/G1. (66)

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(21)

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(12)

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Details and application form from The Establishment Officer, The Polytechnic of Central London, 309 Regent Street, London W1R 8AL. Closing date February 8, 1974. Please quote reference N.

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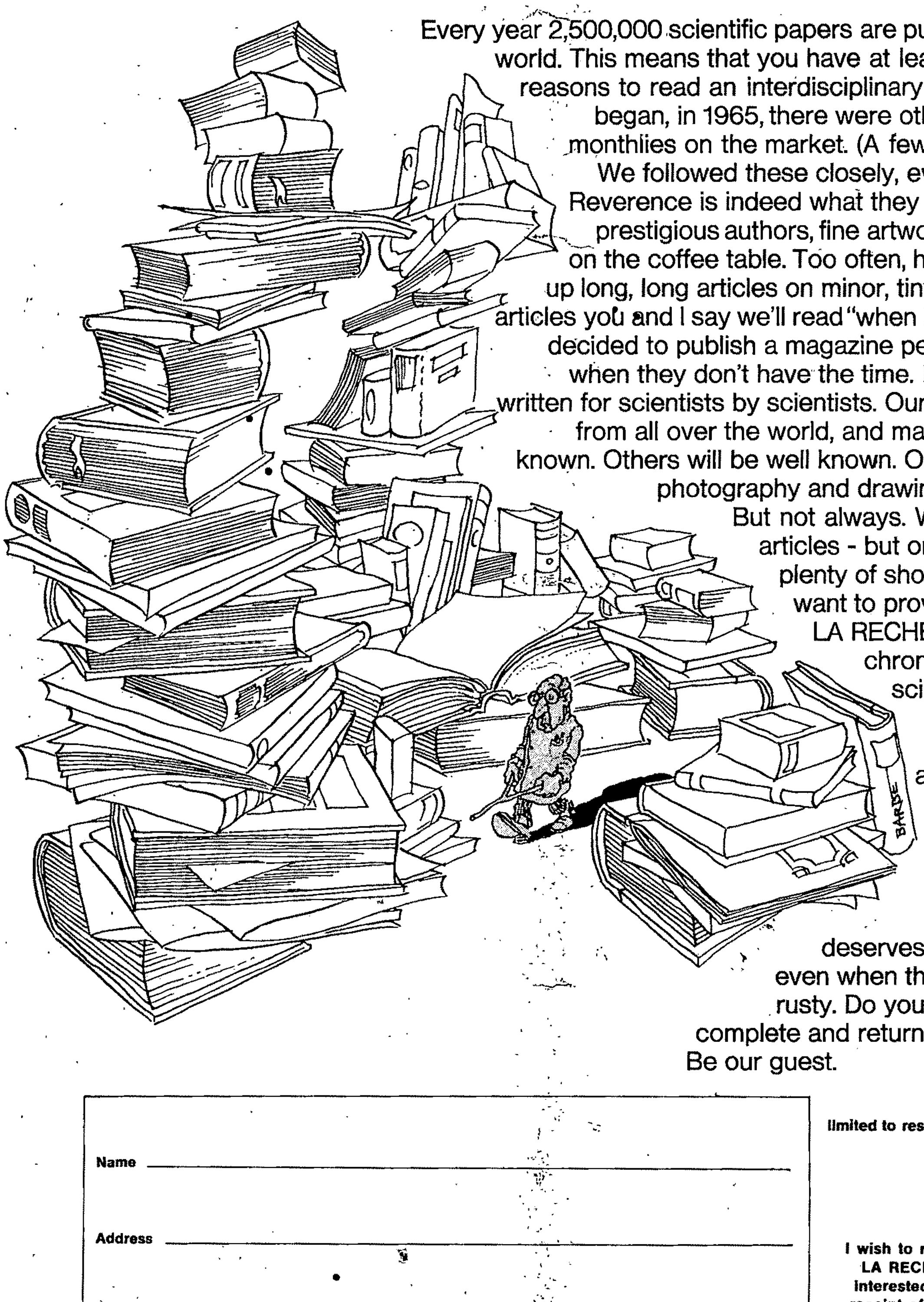

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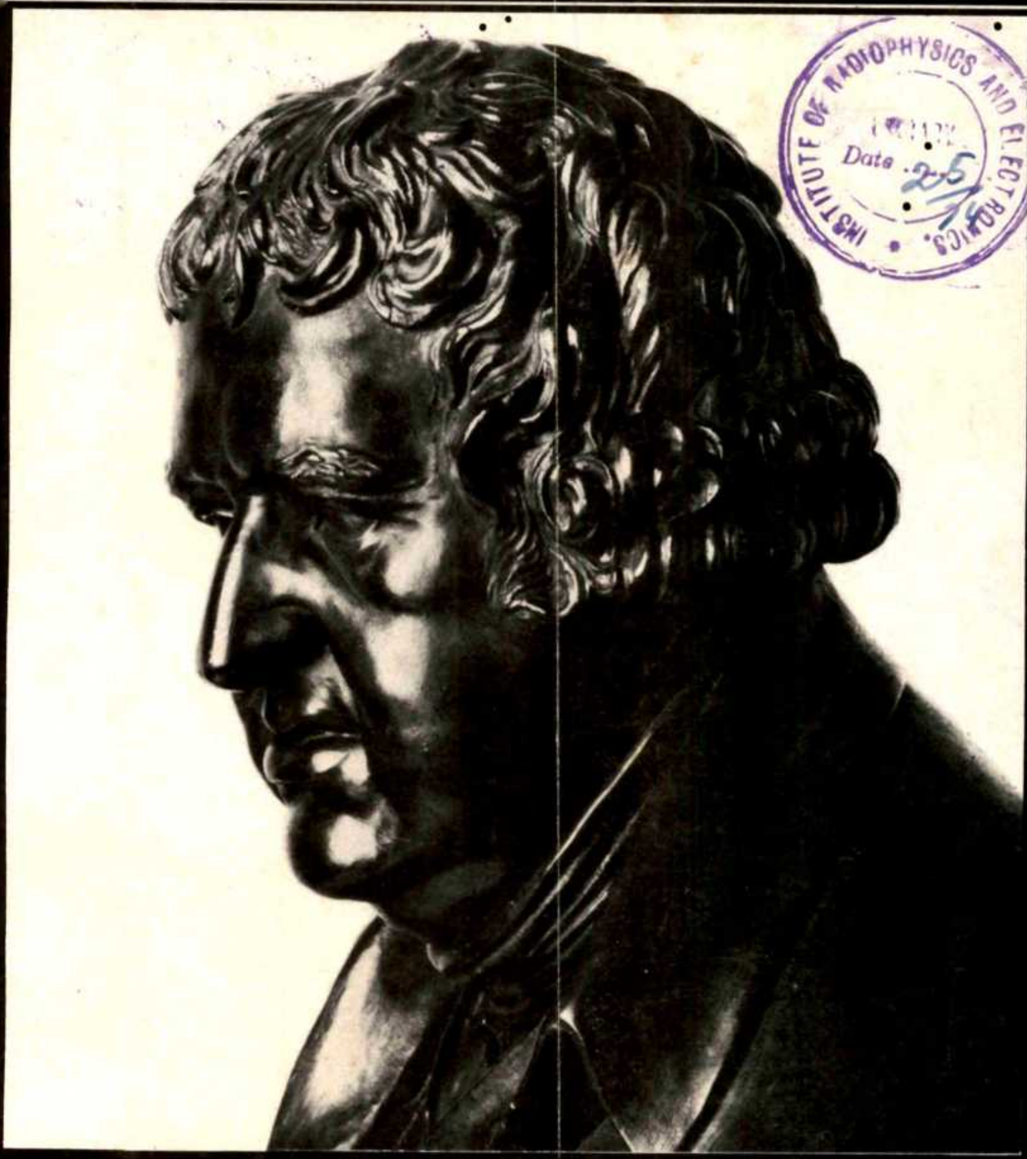

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Erasmus Darwin from a portrait-bust by William Coffee, reproduced by courtesy of Sir Robin Darwin. See also page 87.

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Guide to authors

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Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the Système Internationale. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible. exp (a) is preferred to e^a if 'a' is more than one character. Articles should be accompanied by an abstract of not more than fifty words, and the abstract should list the main conclusions that are drawn.

References are indicated by superscripts in the text. The style may be gleaned from any contemporary *Nature* with the following two changes:

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(ii) The last page as well as the first of any reference should be cited.

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Artwork should be sent with the manuscript. All artwork should be marked with the author's name. Line drawings should preferably be in Indian ink on heavy cartridge paper, although other materials are acceptable; thin, shiny, folded, torn or heavily handled material should be avoided. Matt rather than glossy photographs are preferred. Figures are usually reduced to one column width. The originals should be about twice their final size. Figures, particularly maps, should contain nothing but essential material. It is preferred that the original be unlabelled, but with a copy containing lettering. Labelling on photographs should if possible be avoided entirely.

A fuller guide appeared in *Nature* (**246**, 238; 1973).

WE regret that as a result of the imposition of a three-day working week on industry in Britain it has not been possible to produce a *Nature* of more than 64 pages this week. The crisis shows no sign of abating in the near future and so next week's issue will also be of 64 pages. The 25th January issue, however, is planned to be up to 96 pages and thereafter we hope to be able to produce at least this number of pages each week for the duration of the present emergency.

The London office continues to be open five days a week, albeit candlelit on two of them, and manuscripts are being handled normally.

Mr John Hall has been appointed News Editor of *Nature*. A former London staff member of *The Guardian*, Mr Hall was until recently a freelance writer supplying feature material to *The Guardian Weekly* and other newspapers and magazines in London and abroad.

nature

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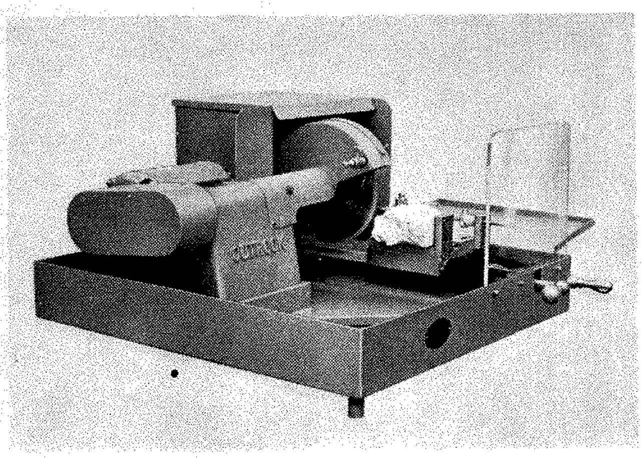

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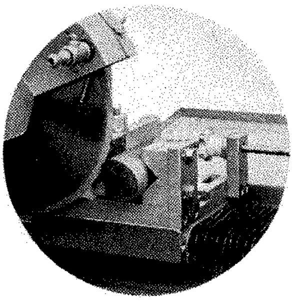

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nature

Volume 247 January 11, 1974

Crisis leads to change

THE outlook from every capital city must at present be bleak and yet nowhere can the immediate prospects be more depressing than in London. Although the rise in the price of oil and restrictions on its supply have hit all countries and the long-term problems of price are terribly serious for developing countries, the industrialised nations are the first to feel the pinch. Britain with its natural gas and coal resources should be marginally better equipped than most other European countries to cope with oil shortages, but it has contrived to compound shortages by virtue of two industrial actions—of the coal miners and of railway engine drivers.

The miners present the bigger problem. Their action has the deeper impact on the country over the longer term and the first effects are now being seen. There is little doubt, despite the inevitable and rather silly political sparring, that coal supplies were falling to a dangerous level and electricity supplies would have been jeopardised by early February. To avoid this the government has imposed a three-day working week throughout industry. The industrial chaos that this is rapidly leading to is deemed necessary in order to maintain vital services up to the end of the winter. It is obvious that one of the purposes of the three-day week has been to prevent coal supplies being run down, as they were in 1972, to a level at which the miners could strike and get exactly what they wanted. There is considerable public sympathy for the miners, whose pay structure contains glaring anomalies.

The government meanwhile pursues its implacable course. Stage Three of its anti-inflation policy, originally heralded as offering flexibility in the negotiation process, is only three months old and seems to offer no negotiating possibilities. Unions have determined to ignore Stage Three in putting in wage claims and the situation is deadlock—so much so that to the exasperation of the public, meetings between the sides are rare, as there is nothing to talk about. During this sleep of reason, the participants seem to have allowed themselves to be saddled with two burdens which sensible negotiators could have avoided—lack of fall-back positions and problems of serious loss of face in the event of failure.

As a result of this, whereas all countries are experiencing hardships, Britain is in danger of falling apart.

The political scene will perhaps emerge as the most damaged. For the past two or three years politicians the world over have been dismayed by the rise in world commodity prices which has taken from them much of their ability to control national economies. This in itself need not discredit the politicians provided a certain amount of frankness is maintained about what governments can and cannot do. Frankness is not, however, a mark of the British politician. Political posturing over inflation has now also become posturing over energy and the three-day week. There is a growing disbelief in the country that either major political party can be turned to in a crisis like this for a cool and lasting solution.

Socially, the danger signals are beginning to appear. The longer-term consequences of the present crisis—a depressed standard of living, less expenditure on desirable projects and so on—are not yet at hand but the short-term indicators, such as the way people behave, are ominous. Just as the bad economic times of the 1920s and 1930s left a mark on the personality of a generation, so the present trauma of crisis, confrontation, divisiveness and inflation may leave a scar of bad temper and distrust.

The military impact of the energy crisis for countries such as Britain lacking local oil supplies is worth comment. It does not seem likely that any such nation will be able to conduct warfare which is displeasing to oil producers without being quickly throttled. The contrast with Suez in 1956 when the retaliation was short-lived is striking. If the crisis thus simply imposed more restraints on military ventures it could be reckoned a good thing. Power to prevent, however, also implies power to promote. Only time will tell whether oil producers can dictate the military activities of other nations.

What are the prospects for science? As we report elsewhere the first impact of budget cuts is now being felt in research spending. This budget was produced before it was clear that the three-day week was a relatively long-term affair. Another month of this and further cuts in public spending may be necessitated. Customer-oriented science is probably a less attractive target for cuts than pure science. Mr Barber's razor may do some of Lord Rothschild's work.

100 years ago

NATURE

THE POLLUTION OF RIVERS

The question which has to be settled is not whether anything is to be done to remedy the certainly disgraceful state of some of our streams, but rather to what extent can the purification be pushed without detriment to the industry of the district; and when this has been decided comes the next question, how this partial purification is to be effected. That it can only be a partial purification is clear from the conclusions of the Commissioners themselves, who do not propose any plan by which the water of our rivers, in populous districts at present little better than sewers, shall be so purified as to be fit for drinking purposes.

From *Nature*, **9**, 197, January 15, 1874.

international news

Energy planning Soviet style

by our Soviet correspondent

ENERGY has been an emotive concept in the Soviet Union since 1920, when the Eighth All-Russian Congress of Soviets adopted the "Goelro" plan for the electrification of the country, with Lenin's famous equation "Communism =Soviet Power+Electrification". It was a coal miner called Stakhanov who, in 1935, by mining fourteen times the normal output of one man in a single shift, became the eponymous hero of the concept of 'Stakhanovite' labour. New prestige projects, such as the Bratsk power station or the Novopolotsk oil refinery, remain standard subjects for poets of a high literary reputation—and their works are analysed by the critics with more than usual attention to their political content. Yet the very significance of energy as a touch-stone of Soviet progress makes it somewhat difficult to assess the overall situation. When the opening of each new power station is reported by the press in careful detail, and with the Soviet propensity for presenting statistics not in absolute figures, but as percentages relative to some inaccessible base year, it is easy to become lost in a mass of minutiae on the one hand and of vague generalities on the other.

Conversely, it is not always easy to distinguish the economically viable project from that undertaken primarily for prestige purposes. Thus the plan described in an *Izvestiya* interview in January 1971 by Minister of Electrification Petr Neporozhnyi, which involved the use of power stations on the White Sea to fill in at peak consumption periods, loses a certain amount of its credibility when one remembers that they would be inoperable during the winter months—at precisely the time when the new unified grid has to depend almost entirely on thermal generation since the Siberian hydroelectric stations are also icebound. On the other hand, the imaginative proposals of I. V. Shishkin for running an entire city on thermal waters would seem to be economically viable, given his basic assumptions of a stratum temperature of 150° C and a sufficient water table (*Priroda*, No. 5, 95–103; 1969).

The present power difficulties of the western nations, however, have evoked considerable comment in the Soviet press from which the picture does become a little clearer. Side by side with cartoons of the pathetic British motorist walking his car to work emerge more factual statements, such as an interview (*Novoe Vremya*, No. 48, 20–22; 1973) with Ruben Napoleonovich Andreasyan, a senior researcher at the Institute of World Economics and International Relations. Andreasyan categorically denied Western reports of an oil shortage in the Soviet Union and was prepared to quote figures. The Soviet Union, he stated, "is one of the richest countries in the world as regards resources of liquid fuel. . . It should be said that the oil of Western Siberia is outstanding not only on account of its high quality, but also its low cost. . . The Soviet Union is already one of the largest oil exporters in the world. In 1972, it exported 76.2 million tonnes of crude oil, not counting prepared petroleum products". As to the reports that the Soviet Union imports Arab oil, this is true, but the amount involved is only 7.8 million tonnes, ten times less than the export figure, and is intended as a gesture of aid to the balance of payments of the oil exporting countries. Of the oil exported, some 50 million tonnes goes to the non-Communist world, and the rest to the Comecon bloc through the "Druzhba" pipeline system, which celebrated its tenth anniversary on December 23, 1973. Reports of the economic integration of Comecon would suggest that by now the smaller member countries supplement their domestic oil production (if any) entirely from Soviet sources, but Reuter reports from Warsaw early in the current crisis (November 25) revealed that Poland, at least, still buys a significant amount of her oil on the world market and has now introduced a speed limit of 80 kilometres an hour and similar measures for saving fuel.

Soviet oil production figures for January to November 1973 (*Pravda*, December 11, 1973) totalled some 385,000 million tonnes (25 million tonnes more than for the same period in 1972), together with some 46,000 cubic metres of natural and petroleum gas. The pride of the Soviet oil industry, the West Siberian (Tyumen') oil field (opened up in 1964), has already surpassed its target for the current year by some 2.3 million tonnes and is expected to produce 87.5 million tonnes in all—some 3 million tonnes above the target. According to a Tass report headlined by the Minsk newspaper *Zviazda*, the Tyumen' field has achieved a record output of 285,000 tonnes in 24 hours.

Nevertheless, the budget and state plan for 1974 (*Pravda*, December 13, 1973) places great stress on "the fuel and energy sectors". The output of electrical power is assigned a target of 975,000 million kilowatt hours (kWh)—an increase of 6.6% in comparison with 1973. The output from nuclear power stations is to be increased to 161,000 million kWh (38% more than in 1973). This increase in electrical output will permit industry to increase its consumption by 5.7%, agriculture by 13% and "the community and living needs of the urban population" by 8%.

The following increases in fossil fuel outputs are envisaged: petroleum and gas condensates by 30 million tonnes (7%), gas by 20.8 million cubic metres (1.8%), mainly from West Siberia, the Kazakh SSR, Central Asia, the Komi ASSR, the Udmurt ASSR and the Perm' and Orenburg regions. To serve these fields, more than 9,000 km of new pipeline and 85 pumping and compressor stations are scheduled to go into service during 1974. The oil refining and petrochemical industries are assigned an increased target of 6.8% with a "considerable increase" in the output of high-octane and low-sulphur fuels. A mouthwatering picture to the harassed Western consumer, perhaps—yet it must be recalled that one has no clear picture of the potential demand, and plans for such large increases may be evidence of the need to close even greater gaps.

Coal output is planned to be 679.1 million tonnes—10 million tonnes more than 1973, with 8 million tonnes of the increase coming from open-cast mining. This emphasis on open-cast mining became evident in the late 1950s with the opening up of new coalfields in northern Kazakhstan and central Siberia.

These figures, it is stated, should basically cover all "normal regular demands". Nevertheless, "it is necessary to exhibit care in expending fuel resources, and to ensure the unconditional fulfilment of the plans for saving fuel and electricity"; this is another indication, perhaps, that demand still outstrips supply.

Geothermal energy in California

Colin Norman, Washington

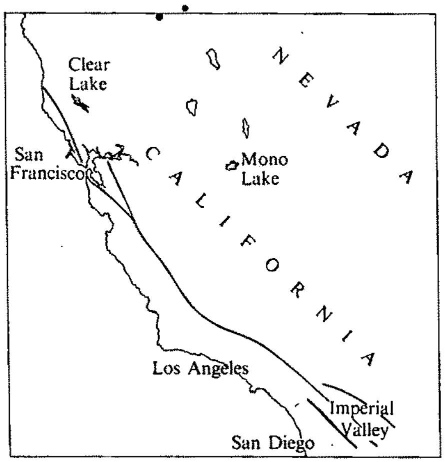
LATER this month the US Department of the Interior will take a large step towards opening up a new source of energy in the United States. On January 22 some 50,000 acres of federal land in California will be leased to private industry so that a start can be made on producing electricity from steam and superheated water trapped in deposits relatively close to the surface. Such deposits of geothermal energy have long been touted as potential sources of power, and the energy crisis has provided the necessary incentive for the federal government to set aside environmental and other concerns and open up large areas of the western United States for drilling to tap the resources.

The potential impact of geothermal energy on power supplies in the United States is a matter of some debate, for estimates range from 1% of energy supplies by the year 2000 to a staggering 20%. A report put together by the University of Alaska under the direction of Walter J. Hickel, former Secretary of the Interior, came up with the prediction earlier this year, for example, that geothermal energy could provide 1,000 million megawatt hours (MWh) of electricity by 1985, and 3,100 million MWh by the end of the century—more than five times the present power demand of the whole of New England. The Department of the Interior, on the other hand, reckons that by the year 2000, geothermal energy may be supplying a modest (but important) 2% of the United States national electricity generating capacity.

In any case, commercially exploitable geothermal resources are regions where the great heat of the Earth's interior is close to the surface. Essentially, that means along the edges of plates and near recent volcanic formations. Since two plates meet at the San Andreas fault in California, and since much of the Western United States consists of volcanic rocks, the potential geothermal resources could be large.

The idea behind production of electricity from geothermal resources is simple enough—in fact, a geothermal power plant has been operating at Larderello in Italy since 1904 and another plant has recently been built in northern California. The easiest deposits to exploit are entrapments of hot, dry steam, which can be tapped simply by sinking a pipe into the deposit and using the steam directly to drive turbines. Deposits of superheated water at about 200° C are also relatively easy to tap, since the water can be flashed to steam by relieving some of the pressure.

Unfortunately, however, such deposits are relatively few and far between, and geothermal energy will have a large impact on power supplies only if methods can be found for exploiting cooler water deposits and hot rock reservoirs. As far as the former is concerned, it may be possible to transfer heat from the water to a more volatile liquid and use the vapour from the second liquid to drive a turbine in a closed system. Hot rock reservoirs are by far the most abundant geothermal resources but they are also the most difficult to exploit. Research is under way on methods which involve fracturing the rock underground with explosives and circulating a liquid through the fractured rock to pick up some of the heat. But commercial application of such techniques is considered to be a long way off.



Map showing locations of areas to be leased.

The areas which the Department of the Interior will lease this month all contain steam and superheated water deposits and thus can be used to generate electricity fairly quickly. Three areas will be leased. One, the Clear Lake–Geysers area in northern California, is already producing some 400 MW of electricity and is reckoned to be capable of producing between 1,000 and 5,000 MW. The second area is in the Mono Lake–Long Valley region east of San Francisco; this is expected to produce about 1,750 MW. And the third area is the Imperial Valley, in southern California, along the Mexican border. This field has the largest potential, for it has been estimated that it could produce between 3,000 and 30,000 MW of electricity, and it also lies close to Los Angeles and other large urban centres.

In addition to opening up those areas for geothermal power production, the Department of the Interior has announced that it will accept bids from private industry for other federal lands which may be potential geothermal resource areas. This should stimulate the search for other areas of geothermal energy which can be easily exploited. And, for the longer term, the federal government is preparing to step up spending on research and development on production of power from less accessible geothermal resources. The five-year plan put together by Dr Dixie Lee Ray, Chairman of the Atomic Energy Commission, envisages an expenditure of some $185 million before 1980, for example.

Agreement on budget for HEW

Colin Norman, Washington

CONGRESS and the Administration have at last managed to reach agreement on the size of the budget for the Department of Health, Education and Welfare (HEW). The agreement will give the research institutes of the National Institutes of Health at least $1,723 million this fiscal year—nearly $200 million more than the Administration had wanted to spend. A number of recent court decisions have also sharply reduced the Administration's ability to hold back money appropriated by Congress and thus it seems unlikely that the Administration will be able to follow its usual tactics of simply refusing to spend extra money voted by Congress.

The compromise budget was passed by Congress early last month and it was signed into law by President Nixon just before Christmas. The fact that a budget has at least been agreed upon is a landmark, for HEW has been operating for more than 18 months on a complicated budgetary formula because Congress and the Administration have repeatedly failed to see eye to eye on spending priorities. Last year, President Nixon vetoed two HEW appropriations bills passed by Congress because he considered them to be inflationary and this year he threatened to veto an earlier version of the bill for the same reason.

Congress has now worked out a formula for the HEW budget which President Nixon still believes to be excessive but which he found politically acceptable. Essentially, Congress added some $1,100 million to the Administration's budget request but it has also given President Nixon power to withhold up to $400 million of the appropriate funds. An important aspect of the bill is that Nixon can lop no more than 5% off the funds allocated to any given programme. Thus congressionally determined priorities cannot be upset by the White House.

As far as the National Institutes of Health are concerned, even assuming that President Nixon exercises his authority to reduce the total HEW budget by $400 million (as he almost certainly will), every institute is set for at least a moderate budget increase. The National Cancer Institute will get $523.6 million—an increase of $23.6 million over the Administration's budget request—and the National Heart and Lung Institute will receive $287.8 million. The largest increase, however, will go to the National Institute of General Medical Sciences, which is set to receive at least $167.9 million; the Administration had wanted to spend only $138.5 million. The National Institute of General Medical Sciences is primarily responsible for supporting basic research and Congress decided to give it a considerable increase after a string of influential scientists had complained that the crusades against cancer and heart and lung diseases were taking money away from important areas of basic research.

Research cuts irksome but tolerable

John Hall

ALTHOUGH cuts in British government spending will cause reductions of more than £8 million (5.5%) in research council budgets for next year, there is a feeling that the economies required are irksome rather than seriously debilitating for the five councils involved.

The Department of Education and Science, which was to have spent £151.6 million (at 1973 values) on research and postgraduate training through the research councils during 1974–75, has issued a revised estimate of £143.5 million, following the chancellor's power crisis budget of December 17, when an overall reduction in public spending of £1,200 million was announced.

The Science Research Council (SRC), which was to have received £72.4 million, will now be given £2.6 million less; the Medical Research Council (MRC), due to have received £29.5 million, loses £1.3 million; the Agricultural Research Council's £23.9 million is cut by £1.4 million; the Natural Environment Research Council (NERC) loses £1 million from its projected £19.5 million; and the Social Science Research Council drops £1.8 million from a proposed £6.5 million.

The NERC is postponing work on two capital expenditure programmes (the Institute for Marine Environmental Research at Plymouth and the British Antarctic Survey building at Cambridge) but otherwise the councils are talking in terms of careful, across the board economies rather than of halting projects which are already under way.

The Agricultural Research Council gave an assurance that salaries and wages of existing staff would not be affected by the 16.3% cut it has to make. The reduction will be spread almost equally across capital and current expenditures, and the effect of the budget changes on individual research units has yet to be determined.

The NERC's saving will be of £600,000 on current and £400,000 on capital spending, and will be achieved by a process of "slowing things down rather than actually cutting anything out".

An MRC spokesman's comment on the cuts was that £1.3 million might look like a lot of money but spread across its own 80 research establishments, and other users, it did not exactly amount to a crippling threat. In the same vein the SSRC promised that "such a small amount" could be saved by marginal reductions in the funds that were made available for new commitments in research and postgraduate training.

The SRC was keeping quiet about the cuts until its members could meet for discussions.

Knights Bachelor

IN the New Year Honours List the following have been created Knights Bachelor:

Stanley George Hooker, Group Technical Director of Rolls-Royce (1971) Ltd;

Maurice George Kendall, for services to the theory of statistics;

John Cowdery Kendrew, for services to the Ministry of Defence;

Charles William Oatley, Emeritus Professor of Electrical Engineering, Cambridge University;

Hugh Norwood Robson, Vice-Chancellor, Sheffield University;

Frederick Henry Stewart, Regius Professor of Geology, Edinburgh University.

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correspondence

Ethiopian Calamity

SIR,—In the article 'The Ethiopian Calamity' (*Nature*, **246**, 53; 1973) your correspondent writes that a plea went to the World Food Programme but that "aid did not materialise from this source for several months".

After almost 11 years of food aid activities during which time this Programme has shipped some four million tons of food aid for social and economic development and emergencies, we have not found it possible to procure and deliver large quantities of food from ports in North America or Western Europe to developing regions in a matter of days or even weeks. This difficulty was fully appreciated by the Government of Ethiopia which, in its request to WFP for 20,000 tons of grain in April this year, asked for delivery by December 1973. Supplies are now arriving as planned. However, because of assurances of the arrival of WFP food, the Government was able to borrow from its national stocks and began distribution in May. The WFP food now arriving will be used to replenish these stocks.

Meanwhile, the Programme has arranged for shipment of a further 15,000 tons of grains plus an additional $350,000 for internal transportation of relief supplies regardless of their source. This will bring WFP's total contribution to 25,000 tons of food grains and $550,000 in cash.

Yours faithfully,

FRANCISCO AQUINO

Executive Director,
World Food Programme,
Via delle Terme di Caracalla,
00100–*Rome*

Fortuitous Correlations?

SIR,—The article 'What's in a Name?' (*Nature*, **246**, 385; 1973) pointed out a number of significant correlations but ignored the rich contributions made by brain research workers to this field[1–4].

Yours faithfully,

G. CURZON

Institute of Neurology,
Queen Square,
London WC1N 3BG

[1] Looney, J. M. (A Biochemical Study of the Blood in Mental Disorders), *Am. J. Psychiat.*, **4**, 29 (1924).

[2] Brain, W. R. (Mind and Matter), *Lancet*, 864 (1951).

[3] Brain, W. R. (Henry Head: The Man and His Ideas), *Brain*, **84**, 529 (1961).

[4] Boring, E. G. (A History of Introspection), *Psychol. Bull.*, **50**, 169 (1953).

news and views

Narcotic action at the molecular level

Opium and its derivatives have been used for centuries to ease the pain of injury and illness, and to blur the mental anguish of society's less fortunate. Morphine, the primary active ingredient of opium, has long been the standard analgetic. Morphine's medical usage, however, is constrained by the body's tolerance to opiates. Ever increasing dosage is required to maintain the analgetic effect. With chronic usage the body becomes dependent on the presence of opiate, and withdrawal causes agony.

For several decades scientists have sought a morphine analogue which would produce analgesia without tolerance (see Eddy and May's review in *Science*, **181**, 407; 1973). Attempts to elucidate the mechanism of opiate action have been spurred on by the epidemic of heroin (diacetylmorphine) abuse which has became a socio-medical problem of staggering proportions in most western cities.

Last spring Pert and Snyder announced their biochemical demonstration of a receptor in nervous tissue which binds opiates with stereospecific selectivity and with affinity paralleling pharmacological potency (*Science*, **179**, 1011; 1973). In the ensuing months a stream of reports from Snyder's laboratory has demonstrated the utility of their assay, both for studying the basic pharmacology of opiate action and for rapid screening of narcotics with medical potential.

In *Nature* on October 26, Kuhar, Pert and Snyder (**245**, 447; 1973) described detailed mapping of the concentration of opiate binding sites in both human and monkey brains. They discovered striking regional variation in binding, with receptor concentrations varying more than thirty-fold between the highest and the lowest areas. Interestingly, the regions high in opiate binding all lie within the limbic system. Binding is greatest within the anterior amygdala, uniformly high throughout the hypothalamus, and high in medial but not in lateral thalamus. Frontal cortex areas show moderate binding of opiate, but little is found elsewhere in the cerebrum. This distribution leads to intriguing speculation about how the analgetic and euphoric effects of opiates might be related to limbic involvement in perception and expression of emotion and in the direction of motivated behaviour.

How well does the density of opiate binding sites correspond to the locations at which externally administered narcotics affect the brain? Previous workers have inserted morphine into various brain regions of living animals and tested for analgetic response; the distributions of opiate binding and of pharmacological effect seem well correlated.

There has been considerable controversy over whether opiate action is associated with some particular neurotransmitter. From the evidence various proponents have presented, one may conclude that the target of opiates is serotoninergic, or noradrenergic, or perhaps cholinergic. In this study, Snyder's group asked whether opiate binding could be correlated with distributions of acetylcholine, catecholamines, GABA or serotonin. Disappointingly, although there were some similarities, there was no clear-cut parallel in any case. Furthermore, lesions of well known nerve tracts specific for acetylcholine, noradrenaline, or serotonin produced no change in opiate binding in the affected areas.

Much narcotic research has centred on searches for the basis of the contrasting actions of opiate agonists and opiate antagonists. Many modifications of the morphine chemical structure have been studied in search of an analogue with a more amenable spectrum of pharmacological activity. In the process of searching, there were discovered certain analogues which block completely the action of opiate agonists (structures with morphine-like action). These 'antagonists' do not cause dependency, and in fact dramatically precipitate withdrawal in morphine-dependent animals. Safe, long lasting opiate antagonists are avidly being sought, with the idea of 'treating' addicts by thwarting the effect of heroin consumption.

Pert and Snyder have recently shown that the various opiate agonists and antagonists have a wide range of binding affinities for nervous tissue, and that there is a high correlation between affinity and pharmacological efficacy of these drugs *in vivo* (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 2243; 1973). Agonists and antagonists apparently compete for the same receptors, and because there is complete overlap in the range of affinities of the two classes of opiate, it would seem that the difference in their pharmacological effect must lie in some difference in cellular response ('intrinsic action') due to contrasting structure. Pert, Pasternak and Snyder have now discovered a difference in the way agonists and antagonists are bound by brain tissue, and that this differential effect can be used to discriminate *in vitro* between the two classes of opiate (*Science*, **182**, 1359; 1973). They first found that injection of mice with either opiate agonists or antagonists produced within minutes a significant increase in the number of opiate binding sites. The narcotic antagonists were 10 to 1,000 times more potent than structurally related agonists in enhancing receptor binding, which corresponds well to the fact that antagonists affect the body at much lower concentrations than do agonists.

In the light of the far greater pharmacological potency of narcotic antagonists, it seemed puzzling that agonists, such as morphine, oxymorphine and levorphanol, showed *in vitro* binding affinities quite similar to those of the structurally corresponding antagonist derivatives nalorphine, naloxone and levallorphan (respectively). This puzzle was resolved by the propitious discovery that adding sodium to the assay medium (in concentrations corresponding to those found in the body) considerably increased antagonist binding, while lowering that of agonists. Interestingly, the increase in binding is caused not by a change in receptor affinity but by an increase in the number of receptor sites available.

The discovery of this differential ionic effect on binding offers the prospect of a simple biochemical test for discriminating the agonist and antagonist activities of drugs which might be used for treatment of heroin addiction or which might provide analgesia without physical dependency. The authors examined the ability of a series of known opiate agonists and antagonists to inhibit the binding of tritium-labelled naloxone (an antagonist), both in conditions of low sodium and of high sodium. High sodium did not alter the binding of antagonists, but drastically lowered the ability of agonists to compete for receptor sites. Pentazocine, which has a mixture of agonist and antagonist properties, showed an intermediate loss of binding. Such mixed action drugs are clinically important because they offer the possibility of 'non-addicting' analgesia. It is theorised that the ability to block pain is a function of agonist affinity, and that addiction is prevented when agonist and antagonist actions are present simultaneously.

K. D.

Histone mRNA and maternal message

from a Correspondent

HISTONE mRNA not surprisingly may be isolated from many cell types as a 7–9S component which resolves on acrylamide gels into six or seven bands (Weinberg *et al.*, *Nature*, **240**, 225; 1972). Until recently the evidence in favour of these RNAs being truly histone messages was circumstantial, but Jacobs-Lorena, Baglioni and Borum (*Proc. natn. Acad. Sci. U.S.A.*, **69**, 2095; 1972) have provided proof that a 7–9S RNA species is translated into histones in an ascites cell free system. The original work of Robbins and Borun (*Proc. natn. Acad. Sci. U.S.A.*, **57**, 409; 1967) showed that in HeLa cells histones are synthesised during S phase on small cytoplasmic polyribosomes and this led to the identification of a rapidly labelled 9S RNA species associated with them (Borun, Scharff and Robins, *Proc. natn. Acad. Sci. U.S.A.*, **58**, 1977; 1967).

Early work by Kedes and Gross on sea urchin embryos (*Nature*, **223**, 1335; 1969; and *J. molec. Biol.*, **42**, 559; 1969) confirmed the existence of a newly synthesised 9S RNA species associated with small polysomes during the phase of rapid cell division called cleavage at the start of development. The system was notable for the very high specific activity to which the 9S RNA could be labelled, for the apparent ease of 9S RNA purification and for its freedom from contamination by labelled ribosomal RNA, which is not synthesised in appreciable quantities until much later. It is not difficult to think of things to do with highly labelled mRNAs even though they lack authentic credentials and, first, Kedes and Birnstiel (*Nature new Biol.*, **230**, 165; 1971), in what they regarded as the first steps to the isolation of a eukaryotic gene coding for a specific protein, produced evidence for reiterated, closely clustered histone coding sequences potentially separable from the rest of the genome.

Weinberg, Birnstiel, Purdom and Williamson (*Nature*, **240**, 225; 1972) took this analysis further by showing that component species separable from 9S histone messages each hybridise to DNA sequences several hundred times repetitive. The different groups of repetitive histone DNA seem to be closely linked since hybridisation of the RNA subfractions to DNA banded in CsCl shows that they all fall in the same region at a density (1.705 g cc^{-1}) just greater than the main band (1.697 g cc^{-1}).

In spite of the fact that histone 9S mRNA synthesis starts soon after fertilisation in sea urchins, treatment with actinomycin D at concentrations which inhibit synthesis permits many cleavages and apparently normal development to the blastula stage (Gross and Cousineau, *Exptl cell Res.*, **37**, 368; 1964). Embryos thus treated continue to synthesise proteins though not nuclear proteins during this time (Raff, Greenhouse, Gross and Gross, *J. cell Biol.*, **50**, 520 ; 1971) and this was regarded as indirect evidence for the absence of stored histone message in unfertilised eggs.

Two recent papers from Gross and his colleagues now provide direct proof that histone mRNA on the contrary is stored in unfertilised sea urchin eggs although at a lower level than that attained during cleavage, and this constitutes the first demonstration of a maternally-inherited message with a defined coding sequence. Many previous attempts have been made to provide direct evidence for stored maternal mRNA in echinoderm (Slater and Spiegelman, *Proc. natn. Acad. Sci., U.S.A.*, **56**, 164; 1966) and *Xenopus* (Davidson, Crippa, Kramer and Mirsky, *ibid.*, **66**, 379; 1972) embryos, but in none of these cases was an identifiable maternal RNA species shown to stimulate the synthesis of a defined product.

What Gross *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2614; 1973) did was to extract RNA from different regions of a sucrose gradient in which a post mitochondrial supernatant from homogenised unfertilised echinoderm eggs had been centrifuged such that the 80S ribosomes were almost pelleted. They found that a mouse ascites cell free system will make protein from these RNA preparations but that the most active fractions are from the ribosome subunit region of the gradient (20–40S). Using aspartic acid and lysine to show up differences in mRNA coding sequence they found that the faster sedimenting RNP fractions give RNA which incorporates more aspartic acid relative to lysine than the more slowly sedimenting fractions. By running the labelled products in acrylamide gels together with authenic labelled histone, and by analysing tryptic peptides, they were able to show that the RNA from a slowly sedimenting (20S) RNP fraction makes histone protein almost exclusively and that the more rapidly an RNP sediments beyond 20S the less histone protein will be made by its RNA. These experiments show that mRNAs exist in non-ribosome bound RNP particles from unfertilised sea urchin eggs and that they are capable of stimulating the synthesis of histone in a cell free protein synthesising system.

Skoultchi and Gross (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 2840; 1973), using the completely different approach of competition-hybridisation to detect specific mRNA sequences, have been able to provide direct support for the conclusions drawn from *in vitro* protein synthesis studies. They used ^{3}H-labelled 9S RNA from sea urchin embryos in conditions in which most of the input labelled RNA can form hybrids with DNA but which still allows identical unlabelled RNA to compete for sites. Both total RNA from small polysomes and unfertilised eggs competed in the reactions but purified 18 and 26S ribosomal RNA, rabbit globin mRNA and 9S histone mRNA from another echinoderm species did not. The curves generated suggest that the amount of histone mRNA in unfertilised eggs is one-quarter of that in the small polysomes of an embryo of 100 cells. This figure is in agreement with similar work by Farquhar and McCarthy (*Biochem. biophys. Res. Comm.*, **53**, 515; 1973), who used very different conditions for their hybridisation.

Skoultchi and Gross went on to show that RNA from subribosomal RNP particles from unfertilised eggs competes with 9S histone mRNA in agreement with the prediction from the protein synthesis studies of Gross *et al.* These papers represent a real advance since they unequivocally identify by two very different methods a messenger RNA which is maternally inherited.

Role of plastocyanin in photosynthesis

from our Photosynthesis Correspondent

THE old argument whether or not plastocyanin acts between cytochrome *f* and the reaction centre of photosystem 1 (P700) now seems to be over. It has been known for many years that this copper containing protein is an important component of the photosynthetic electron transport chain but there have been several contradictory hypotheses about its exact site of action. The most widely accepted view is that plastocyanin acts as an intermediate between cyt *f* and P700. Not all investigators, however, agree with this scheme and prefer to place plastocyanin on the oxidising side of cyt *f* so that cyt *f* acts as the primary electron donor to P700. Alternative suggestions have been that plastocyanin and cyt *f* act in parallel in such a way that they both donate electrons to P700 or that they are associated with the different photosystems; plastocyanin with photosystem 2 and cyt *f* with photosystem 1.

It now seems from a recent publication from San Pietro's laboratory at Indiana (Siedow, Curtis and San Pietro, *Arch. Biochem. Biophys.*, **158**, 889; 1973) that the most popular hypothesis is correct. In a very careful piece of work Siedow *et al.* have demonstrated that plastocyanin acts stoichiometrically as an electron carrier between cyt *f* and P700. Their work is particularly convincing because they have managed to prepare subchloroplast particles which are completely depleted of plastocyanin. These particles were obtained by sonicating osmotically shocked chloroplasts suspended in a medium containing a low concentration of pyridine. Using difference absorption spectroscopy Siedow *et al.* have demonstrated that these particles were unable to support the photo-oxidation of cyt *f* until plastocyanin was added to the suspension. Moreover, they found that it was necessary to either treat the particles with non-ionic detergents such as digitonin or sonicate them after adding the plastocyanin to induce full photo-oxidation of the cytochrome.

The results of these experiments clearly support a strict linear sequence of electron transport on the oxidising side of photosystem 1 from cyt *f*→ plastocyanin→P700. Furthermore, the requirement for sonication or detergent treatment to bring about the full reconstitution supports a concept already put forward by Hauska *et al.* (*J. biol. Chem.*, **246**, 189; 1971) that plastocyanin, *in situ*, binds to a specific site on the inner surface of the chloroplast membrane.

Structural analysis by electron diffraction

from a Correspondent

A JOINT meeting of the Crystallography and Electron Microscopy Groups of the Institute of Physics was held at the Institute of Electrical Engineers, London, on November 15–16 to discuss the use of electron diffraction for structure determination. The contributions covered electron diffraction from gases and surfaces, structural analysis of thin films by combined electron diffraction and microscopy, direct lattice resolution by electron microscopy, as well as applications of these techniques to the study of molecular crystals and minerals. There was also a discussion of the interpretation of electron micrographs and diffraction patterns of amorphous materials.

Gases and surfaces

D. W. J. Cruickshank (UMIST, Manchester) opened the meeting with a review of electron diffraction from gases. Since the 1930s some twenty groups around the world have analysed between them more than 1,000 molecules. The techniques and methods of interpretation are by now fairly standard and the interest lies in the chemistry of the molecules themselves. It is particularly interesting to compare rather similar molecules and to find surprises—for example, $(SiH_3)_3N$ is a planar molecule whereas $(SiH_3)_3P$ is pyramidal. Similarly, in the so-called 'sandwich' molecules $(C_6H_6)_2Cr$ and $(C_5H_5)_2Fe$, the metal atoms are symmetrically placed between the rings but in $(C_5H_5)_2Be$ the Be atom is asymmetrically placed and in $(C_3H_5)_2Ni$ there are two variants, in one of which the two carbon frameworks are not parallel.

M. Prutton (University of York) gave an account of the determination of surface structures by RHEED (reflection high energy electron diffraction) and LEED (low energy electron diffraction). Both of these methods are sensitive to the crystallography of the surface layers because of the low penetration of the electron beam and both can be used to obtain the mesh sizes of the surface and of adsorbed layers. The use of quantitative intensity data to deduce the position of atoms in the unit mesh has, however, proved more difficult. In the case of RHEED, transmission diffraction through asperities presents an experimental problem and surfaces with small roughness on the 10 Å scale are needed. For LEED, although progress in the theory has been rapid recently, Prutton felt that it was still extremely difficult to make good quantitative statements. This point was (perhaps inadvertently) reinforced by M. A. van Hove (University of Cambridge), who indicated that in the case of oxygen adsorbed on (100) Ni in a c(2×2) structure, the oxygen atom has been deduced by different workers to be either 1.5 ± 0.1 Å or 0.9 ± 0.1 Å above the Ni atoms, with an almost equally good fit to theory in both cases, but that values in between were impossible on the basis of a match between dynamical LEED theory and experiment. Of the two possible values the first is much more plausible from a chemical viewpoint.

The information obtainable from an understanding of the dynamical transmission diffraction effects through thin films was reviewed by J. W. Steeds (University of Bristol). Of the two techniques considered, the convergent beam technique and the analysis of electron microscope images of bent crystals, it is the second which is easier to use for qualitative crystal structure determination. Steeds showed that one could easily use the form of bend contours to deduce the point group of the crystal and that dark-field observation could establish the presence of centres of symmetry, glide planes and screw axes, which could lead to the establishment of the space group. Applications to layer structures such as mica and molybdenite were described in this and in the following papers by G. J. Tatlock and Steeds. The simulation of these bend contours using many-beam dynamical theory yielded impressive agreement with the experimental contours, and enabled them to distinguish easily different polytypes of molybdenite and to detect the presence of otherwise invisible stacking faults.

Lattice images

J. Hutchison (University of Oxford) gave a fascinating glimpse into the future with his talk on direct lattice resolution by electron microscopy. In the past few years the electron microscope has been used to study the lattice images of several structures including a class of complex oxides called block structures based on NbO_6 octahedra. The octahedra are stacked together in blocks of, say, 4×3 or 5×3 and have tetrahedral sites at the corner, where four blocks join together, which can accommodate substitutional metal ions. In the extremely high resolution micrographs shown, the blocks and the positions of the heavy atoms in a compound such as $W_4Nb_{26}O_{77}$ were clearly seen, and the experimental images were in excellent agreement with simulated images based on *n*-beam dynamical theory; one can almost resolve the individual octahedra in both the experimental and simulated images. The images depend in detail on the defocus of the objective lens, however, so that it is essential to know the amount of defocus and include this in the calculations.

Quasi-amorphous films

The use of dynamical diffraction theory for transmission electron diffraction from single crystal films is well established and Hutchison and Steeds elegantly showed how well the theory works. By contrast, the best theory for describing the diffraction from quasi-amorphous films is not nearly so clear; this was the theme of a panel discussion between W. Cochran (University of Edinburgh), A. Howie (University of Cambridge) and M. V. Berry (University of Bristol) under the chairmanship of D. J. H. Cockayne (University of Oxford). The problem arises because there are many possible structures of quasi-amorphous solids (such as amorphous Si and Ge) which would give the same diffraction pattern. Can lattice imaging in the electron microscope be used to give extra information? Previous work by Rudee and Howie had suggested the existence of microcrystallites, some 15 Å in diameter, on the basis of groups of 'lattice' fringes observed using a particular part of the diffraction pattern to form the image. These small regions are, however, embedded inside a film of thickness ≃200 Å, so that they are certainly not isolated crystallites, and the interference with the regions above and below any such crystallites, as well as the degree of defocus, are important in determining the image. Cochran has performed simulations of the image of a random network model and considers that although the fringes are unlikely to be due to such a structure, they do not really require a microcrystallite model either. He showed that changes in defocus during the exposure could cause certain periodicities in the image to be enhanced. Berry was inclined to favour an approach based on simple image transfer theory which he claimed means that the fringes in the image were merely random noise, spatially filtered by the objective aperture; provided a relatively small segment of the diffraction ring is used to form the image, groups of fringes would be observed, essentially independent of the detailed structure of the sample. Thus it is clear that the unravelling of the detailed structure of these quasi-amorphous films remains a difficult problem which is far from solved, though it is certain that electron microscopy has a part to play in its resolution.

Molecular solids

The application of electron microscopy and diffraction to the study of molecular solids and minerals was the subject of the last session at the meeting. C. A. English (University of Sussex) reviewed the work on solid rare gases, O_2, N_2 and CO_2, which has been carried out largely at the University of Sussex. An electron microscope fitted with a liquid helium stage is used and single crystals can be produced by annealing the thin films obtained by vapour deposition at a pressure of a few torr. Among the examples he described were the determination of the structure of α-N_2, the measurement of the stacking fault energy of solid xenon, twinning in β-O_2 and radiation damage in solid O_2, N_2 and CO_2.

An elegant structure determination of a molecular solid, poly sulphur nitride, by electron diffraction, was described by M. Boudeulle and P. Michel (University of Lyon). Also, the complexities of graphite-halogen intercalation compounds, including structures with very long period order giving rise to the very beautiful diffraction patterns, shown by W. T. Eeles (UKAEA, Capenhurst), revealed that there is much to be learned about the bonding in all the molecular solids discussed. The advantages of combined use of electron microscopy and single crystal electron diffraction when the crystals are too small for single crystal X-ray work were well illustrated in all these contributions.

Minerals

This same theme was reinforced by P. E. Champness (University of Manchester) in her review of electron diffraction and microscopy as a means of characterising minerals. Analysis of the characteristic X rays by EMMA (combined electron microscopy and microprobe analysis) to obtain chemical information from regions as small as 1,000 Å in diameter is also invaluable. A particularly impressive example of the combined use of these techniques is the study of an igneous pyroxene containing lamellae around 0.1 μm thick spaced about 50 to 100 μm apart. In between the lamellae there are very fine precipitates with a precipitate free zone next to the lamellae. The orientation relationships of all the phases can be determined by electron diffraction. Analysis of the Ca/Si X-ray intensity ratio reveals that the lamellae are rich in calcium and the diffusion profile of the calcium content between adjacent lamellae can thus be determined. The fine precipitates can be imaged by lattice resolution of the 1.8 nm spacing and shown to be only one unit cell thick.

The conference clearly showed the range of opportunities offered by electron diffraction, particularly when it is used in combination with electron microscopy and other techniques such as X-ray analysis. The greater difficulty of interpretation *vis à vis* X-ray diffraction remains, but well-founded dynamical diffraction theory is being used with very considerable success for quantitative work on thin films with high energy electrons. At low energy, theoretical improvements are rapidly being made, but much remains to be done before the theory can be used with complete confidence. Direct structure determination by high resolution electron microscopy is now a fascinating possibility and will undoubtedly be explored further in the future.

If M haemoglobin, then R or T?

from a Correspondent

Much ingenuity has been shown in utilising the structural differences found in the genetically determined haemoglobin variants to assist the study of the relationships between structure and function of normal haemoglobin. A striking example of this approach is the work of Mayer, Ogawa, Shulman and Gersonde (*J. molec. Biol.*, **80**, 187; 1973) on high-resolution proton nuclear magnetic resonance (NMR) spectral studies of the quaternary state of haemoglobin M(Iwate).

Hb-M(Iwate) is an α-chain variant (α^{87}His→Tyr, β_2) and is identical with Hb-M(Oldenburg) and Hb-M(Kanakee). Apart from shortening the list of M-type haemoglobins, the locations in which it has been discovered provide an interesting example of the probable independent occurrence of the same mutation in three widely separated places. As with other M-type haemoglobins the chemical properties of the haems on the variant chains are abnormal. It has previously been shown that in the crystal the quaternary state is deoxy (T) when the normal β-chain subunits are deoxygenated and also when they are oxidised to methaemoglobin and therefore ligated with H_2O. In view of the low oxygen affinity of the variant it seemed probable that after ligation it remains in the T quaternary structure in solution as well as in the crystal. Mayer and colleagues have therefore used Hb-M(Iwate) to test the extent to which three NMR spectral features are linked to the state of the quaternary structure.

These three features are (1) the paramagnetically-shifted resonances of the cyanide-liganded subunits in valency-hybrid haemoglobin, (2) the upfield resonance in oxy and carbonmonoxyhaemoglobin assigned to Val E11 near the haem group, and (3) the low-field resonance near −14 p.p.m. (referred to 2,2-dimethyl-2-silapentane-5-sulphonate) in deoxyhaemoglobin which comes from an exchangeable proton, and which disappears on ligation. Although these three features had been used to distinguish between oxy (R) and deoxy (T) quaternary structures, their validity had not been tested with a haemoglobin known to be in the T quaternary state in solution even when ligated.

A comparison of the 220 MHz NMR spectra of normal human adult haemoglobin valency hybrids and of Hb-M (Iwate) in the presence and absence of organic phosphates clearly shows that those NMR spectral features previously attributed to the deoxy (T) quaternary structure are present in Hb-M(Iwate) when the β subunits are ligated to O_2, CO or CN^- as well as when they are unligated, with the quaternary structure remaining in the deoxy (T) state throughout. These results complement previous spectral studies on Hb-Kansas ($\alpha_2\beta_2$ 102 Asn→Thr) and on valency-hybrid haemoglobin.

In the authors' view, the increasing body of evidence that the quaternary structure is the most important factor in determining the functional properties of haemoglobin provides additional support for a two-state model, as proposed by Monod, Wyman and Changeux in 1965, as a suitable first-order description of haemoglobin function.

In another application of NMR spectroscopy to the study of haemoglobin, Manuck, Maloney and Sykes (*ibid.*, 199) have studied the binding of methyl *iso*nitrile to the β chains by measuring the transverse relaxation times (T_2) of the *iso*nitrile protons as a function of protein concentration, temperature and ^{14}N decoupling. Binding both at the haem iron and at non-specific site(s) affects the measured relaxation times. From the results a value of 57 ± 12 s^{-1} for the 'off' rate constant (k_{-1}) for specific binding, and an Arrhenius activation energy for k_{-1} of 14 ± 3 kcal mol^{-1}, were obtained.

Erasmus Darwin, master of many crafts

Desmond King-Hele

Royal Aircraft Establishment, Farnborough, Hampshire

Erasmus Darwin was famous in the eighteenth century as a physician, an inventor and a best-selling poet. Today he earns most credit for recognising biological evolution, discovering how clouds form, analysing plant nutrition and influencing the English Romantic poets.

DR ERASMUS DARWIN, who lived from 1731 until 1802, achieved more in a greater variety of intellectual disciplines than anyone since. Why then has he not gained the fame he deserves? Partly, perhaps, because we feel that his family already has enough fame, through his grandson Charles; and partly because of our unspoken but deep conviction that no one can really master many different crafts, as Darwin did. He belongs with Leonardo da Vinci and Goethe, immensely capable in practice and theory, in life, in literature and in science.

By profession Darwin was a physician, generally recognised as the finest doctor of his time in England. "Within the narrow resources of 18th-century medicine, he was supreme"[1], but he refused King George III's repeated requests to become royal physician[2]. By nature Darwin was a large and powerful-looking man (Fig. 1), cheerful and healthy, and, despite a stammer, a witty and rather domineering talker. By inclination he was extremely sociable and inventive. With Matthew Boulton and William Small, he founded the Lunar Society of Birmingham[3], which was the real intellectual driving force behind the Industrial Revolution in England and hence the modern technological world[4]. Encouraged by his Lunar friends, especially Watt and Wedgwood, Darwin produced working inventions ranging from a speaking machine to a horizontal windmill, and dozens of designs on paper, from a canal lift to a steam turbine. Darwin won greatest acclaim, however, in a quite different sphere, as a poet: his long poem *The Botanic Garden* took the literary world by storm in the early 1790s. Coleridge called him "the first *literary* character in Europe"[5], the Napoleon of literature, as it were.

Today Darwin earns most credit among scientists for his recognition of biological evolution[6,7], his psychological ideas[8], his analysis of plant nutrition and photosynthesis[9], and his discovery of the main process of cloud formation[10]. And the literary critics are ready to honour him not for his poems but for his immense influence over the English Romantic poets, Wordsworth[11,12], Coleridge[11-16], Shelley[11,17] and Keats[18,19].

Lichfield and the Lunar Society

Erasmus Darwin was born at Elston near Nottingham, the third son of a moderately successful lawyer. He went to Chesterfield School and from there to Cambridge, where he studied medicine and took his BA in 1754. Then came two years at the leading medical school, at Edinburgh. After this thorough schooling in dubious medical theory, he was ready to be loosed on the public. Finding no patients at Nottingham, he migrated to Lichfield, where he had the good fortune—or perhaps skill?—to cure a prominent local man whom all other doctors had condemned to death[20]. His future career assured by this *coup de théâtre*, Darwin settled at Lichfield. From 1758 until 1781, living in the pleasant house which still stands on Beacon Street at the western extremity of the Cathedral Close, he gave medical advice expensively to the rich and freely to the poor.

Today Lichfield is overshadowed by its neighbour Birmingham 10 miles to the south; but in the eighteenth century their roles were more nearly equal, and the Cathedral Close housed a literary coterie, headed by Canon Seward, the Dean, who edited the plays of Beaumont and Fletcher, as if to rival Lichfield's leading literary figure, Dr Johnson. As Darwin put it,

> From Lichfield famed two giant critics come,
> Tremble, ye poets! hear them! 'Fe, Fo, Fum!'
> By Seward's arm the mangled Beaumont bled,
> And Johnson grinds poor Shakespeare's bones for bread[22].

Canon Seward's daughter Anna, who was 14 when Darwin arrived at Lichfield, later became quite famous as a poet, with some help from Darwin: she was known as "the Swan of Lichfield" in insulting analogy with "the Swan of Avon".

In 1758 Darwin married Mary Howard, whose family also lived in the Close; the marriage proved a very happy one, marred only by Mary's continual ill-health. They had three sons who survived infancy. The eldest, Charles, was a brilliant medical student, but he died tragically at the age of 19 from an infection, after cutting his hand while dissecting. The second son, Erasmus, became a lawyer. The third, Robert, followed in his father's footsteps, becoming a successful doctor at Shrewsbury; he was an impressive man, not only in appearance (he weighed 24 stones) but also in ability, and especially in his uncanny power of reading character, much admired by his son Charles[23].

Erasmus Darwin was at his most inventive and socially active during the 1760s. One of his earliest friends was Matthew Boulton[24,25], then a humble manufacturer of buckles in nearby Birmingham. But his energy and enterprise were to make Boulton the chief manufacturer of England and "the father of Birmingham". Darwin's "favourite friend" in the late 1760s was the learned and liberal Dr William Small, recently returned from teaching in America, where Thomas Jefferson, one of his pupils, declared that Small's example "fixed the destinies of my life". About 1766 Darwin, Boulton and Small began those monthly meetings of friends at the time of the full Moon, which were soon to develop into what became known as the Lunar Society of Birmingham.

In the early days of his friendship with Boulton, in 1763, Darwin was madly enthusiastic about the idea of a steam carriage with twin cylinders, which he hoped Boulton would help him to construct. Boulton was rightly cautious, but Darwin's enthusiasm for steam was not wasted: James Watt on his first visit to England in 1767 called on Darwin[25,26] and began a lasting friendship, which benefited both. Watt was rather gloomy by nature and needed encouragement: who better to provide it than the energetic and optimistic steam-enthusiast Darwin? No wonder Watt, though usually reticent,

Fig. 1 Erasmus Darwin from a portrait-bust by William Coffee, reproduced by courtesy of Sir Robin Darwin.

praised Darwin so highly: "It will be my pride while I live that I have enjoyed the friendship of such a man"[27].

Another man who enjoyed that friendship to the full was Josiah Wedgwood[28], who met Darwin when both were about 30. When in 1765 Wedgwood and Brindley began promoting what was virtually a new transport system, the Trent-and-Mersey canal, Darwin was an enthusiastic supporter. Darwin's horizontal windmill was used for grinding colours at Wedgwood's factory for 14 years, until displaced by steam. The Darwin–Wedgwood alliance was strengthened by the marriage of Robert Darwin with Susannah Wedgwood in 1796.

One of Darwin's friends at Edinburgh had been the chemist James Keir[29], who after a spell in the Army settled at West Bromwich in 1768, and later set up an alkali factory at Tipton nearby. Keir was a pioneer of the chemical industry, and his clever process of obtaining soda from salt in dilute solution[30] was commercially successful many years before the Leblanc process was devised.

On his daily rounds as a doctor, Darwin was bumped and joggled over the vile roads in his carriage, so it is not surprising that he was keen to construct comfortable carriages. Wedgwood greatly admired a design which used the wheel spokes as springs (an idea continually being resuscitated, even today[31]) with each spoke shaped in the serpentine line of beauty. A carriage of Darwin's, peculiarly resistant to overturning, greatly impressed Richard Edgeworth[32], and inspired him to become an inventor. He visited Darwin in 1766 and became a lifelong friend.

This group of Darwin's friends—Boulton, Small, Watt, Wedgwood, Keir and Edgeworth—formed the nucleus of the early Lunar Society; Priestley was a later addition to their number. "Intellectually the most effective provincial group that has ever come together in England", C. D. Darlington has called them[6]. Individually they were among the most energetic and intellectually ablest men of the era, and their meetings, by some strange alchemy, channelled their talents fruitfully, producing a unique forward thrust in science and technology.

Middle and later life

During the 1760s Darwin's reputation as a doctor continued to grow, but he had no cure to offer his wife Mary, who died in 1770; her tributes to her husband were carefully recorded by Anna Seward[20]. In the 1770s Darwin remained at Lichfield, unmarried; but he had two illegitimate daughters, known as the Misses Parker.

Then in 1778 he fell in love with Mrs Elizabeth Pole, the lively, young and beautiful wife of a leading Derbyshire landowner, Colonel Sacheverel Pole of Radburn Hall. At first Darwin could only woo her in verse[20], but in 1780 Colonel Pole conveniently died and Darwin, though rather ugly, fat and fifty, brushed aside more eligible rivals and married Mrs Pole in 1781. They moved to Radburn and later to Derby. This second marriage proved as happy as the first, and the seven children of the marriage acknowledged their good fortune in having been brought up in such a happy atmosphere. This quiet happiness seems to have lasted throughout the 20 outwardly uneventful years of Darwin's second marriage[12]. He died in 1802 at the age of 70, of a heart attack.

A flowering of verse

Though outwardly unspectacular, these later years saw the publication of Darwin's five books, each epoch-making in its own way. First, in 1789–91, when he was already 60, came his encyclopaedic poem, *The Botanic Garden*, which gripped the literary world like a fever: "Dr Darwin has destroyed my admiration for any poetry but his own"[33], wrote Horace Walpole, a sentiment echoed by many another hard-boiled literary pundit. Part II of the poem, "The Loves of the Plants", had been published first, in 1789, because it was lighter—968 rhyming couplets about the sex life of plants, nicely personified and backed by masses of scientific notes. Part I, "The Economy of Vegetation", published in 1791, belies its title: it is really a compendium of scientific knowledge in 1,224 rhyming couplets, again with lengthy notes in support. The complete *Botanic Garden*, which was beautifully reprinted in facsimile by Scolar Press last year[34], runs to 550 quarto pages.

Was it wrong of those readers of the 1790s to admire *The Botanic Garden*? The poem is certainly rather rambling and unstructured; but Darwin's polished couplets have a lustre that time cannot tarnish, as generations of literary critics confirm (see, for example, refs 35 to 38). To this skill in verse Darwin added three other talents: 'a greater range of knowledge than any other man in Europe', as Coleridge put it[39]; originality in proposing new theories and foreseeing new technology; and an amazing lightness of touch—he is forever laughing at himself. As an example of all three talents, here is his forecast of mechanically-propelled cars and aircraft:

> Soon shall thy arm, Unconquer'd Steam! afar
> Drag the slow barge, or drive the rapid car;
> Or on wide-waving wings expanded bear
> The flying-chariot through the fields of air.
> Fair crews triumphant, leaning from above,
> Shall wave their fluttering kerchiefs as they move;
> Or warrior bands alarm the gaping crowd,
> And armies shrink beneath the shadowy cloud[40].

When Darwin switches from technology to plants in Part II, comedy is still in the ascendant as he promises to tell us

> What Beaux and Beauties crowd the gaudy groves,
> And woo and win their vegetable Loves.

For example in *Lychnis*, where the males and females are on different plants, each female saucily peeps out when mature:

> In gay undress displays her rival charms,
> And calls her wondering lovers to her arms[41].

Science so pleasantly presented was much to the taste of the leisured reading public. As L. T. C. Rolt has remarked, Darwin performed "the astonishing feat of rendering in imaginative terms which any literate man could then appreciate the sum of human knowledge at that time. It was an achievement which was never repeated or even attempted[42]".

Medicine, evolution, young ladies and plants

In his later years Darwin's fame as a doctor became excessive. With a lifetime's experience and few new discoveries to outdate it, he was a legendary figure, whose every word was eagerly sought—a status scarcely credible today when the mystique of the doctor has largely evaporated. Although his medical theory was unsound, Darwin deserved his fame as a doctor because he was so keenly observant and could often guess correctly the future course of an illness; his cheerful attitude and his sympathy with the patient also had a good effect. To this must be added his long and deep thought about medicine, expressed in the second of his books, the two massive volumes of his treatise on animal life, *Zoonomia*, published in 1794–96. Dr Beddoes called it "perhaps the most original work ever composed by mortal Man", which would "place the author amongst the greatest of mankind[43]". We do not value the book so highly today because its classification of diseases has too many flaws. But we can still admire some of his bold experimental cures, his linking of body and mind, which has been influential in philosophy[8,14], and his pioneer work in treating mental illness humanely instead of brutally. Whatever our reservations now, *Zoonomia* had great influence in its day: it went into a second edition in 1796 and a third (revised) edition in 1801; provoked a 560-page critical commentary in 1798; "was translated into German, French and Italian and was honoured by the Pope by being placed in the *Index Expurgatorius*", as Charles Darwin remarked[44].

Zoonomia is mainly medical, but it has one riveting chapter on biological evolution. As I have explained in a previous article in *Nature*[7], Erasmus appreciated and described the main mechanisms of evolution, and made it part of his habitual thinking for 20 years. In the England of the 1790s, reeling from the shock of the French Revolution, evolution was quite unacceptable: denying that God created species was near-treason; but Darwin was not sentenced to transportation, only to satire. In the *Anti-Jacobin* magazine in 1798 he was ruthlessly lampooned by Canning and others[45], while Coleridge condemned his evolutionary speculation as mere "Darwinizing".

Erasmus Darwin's correct advocacy of evolution does not detract from Charles Darwin's achievements. Rather it helps to explain Charles's success, by bringing out the strong tradition of evolutionary ideas in the family. For Charles's father Robert followed Erasmus in his profession and in his ideas (on alcohol and religion as well as evolution).

Erasmus carried his evolutionary ideas further than his grandson did, weaving it into his philosophy of life. Erasmus saw the full horror of the struggle for existence, more clearly than anyone else in his day, but he saw the bright side too. He believed most creatures delight in life; so, the better adapted they are, the happier they will be. Evolution is, in that sense, the survival of the happiest. Although highly organised animals have a greater capacity for happiness than lowly creatures, the plants and insects by their vast numbers make up a large part of the sum of happiness. This is the basis of his remarkable philosophy of organic happiness. He looks on rocks like limestone, chalk and coral, the remains of once-happy creatures, as "mighty monuments of past delight" and thinks we "should eye with tenderness all living forms". He is very much in favour of sexual love, not only because sexual generation speeded up evolution by increasing variety, but also because sex is a major component of organic happiness—he regards sexual pleasure as the greatest source of delight for lower creatures as well as human beings.

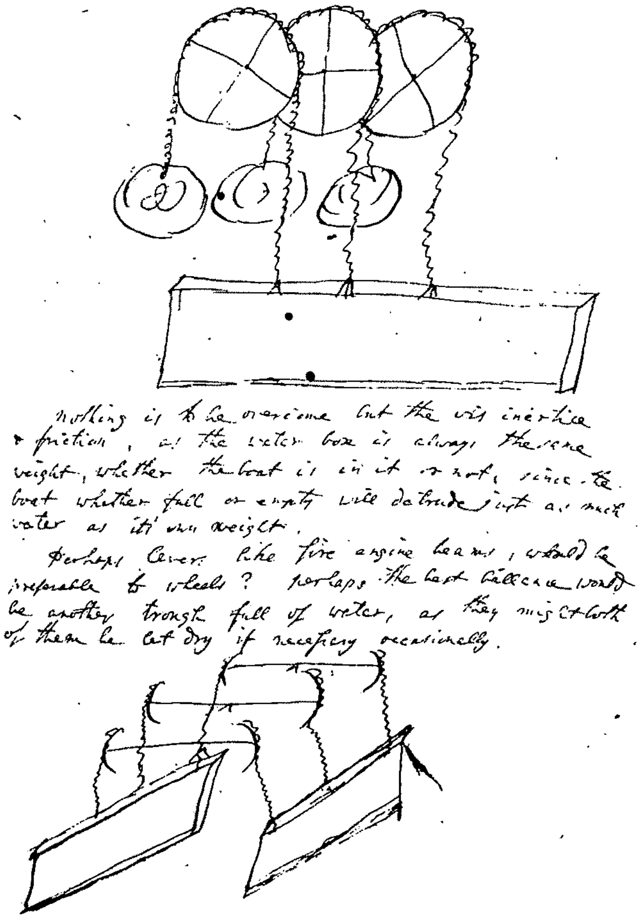
The third of Darwin's books is (for a change) a slim volume of 128 pages, entitled *A Plan for the Conduct of Female Education in Boarding Schools* (1797). Reprinted[46] in 1968, this is probably the most widely read of his books because of its significance in the history of education[47,48]. Darwin totally opposed the conventional idea of his time that girls should become feather-brained simpering misses: he wanted to see them "sound in body and mind". "The advantages of a good education consist in uniting health and agility of body with cheerfulness and activity of mind"[46]. His book, written for his daughters Susan and Mary Parker (who had set up a school), marks a quantum jump in the progress of girls' education, almost approaching modern ideas of sexual equality. Darwin deserves a small memorial in the pantheon of Women's Lib—though ironically the church where he was buried was burnt to the ground by suffragettes in 1914.

The fourth of Darwin's books, *Phytologia* (1800), is an impressive 600-page survey of vegetable life and all that sustains it, from carbon dioxide down to manures. *Phytologia* is a much better book than *Zoonomia* because it has so many ideas that are correct and new. One example is his discussion of plant nutrition, which is a great advance on any previous analysis. He specifies the process of photosynthesis, not of course in one neat equation (CO_2 + water + light energy = sugar + oxygen), but in separated sentences:

"This carbonic gas [CO_2] . . . is the principal food of plants. . . . Next to carbonic gas . . . water seems to afford the principal food of vegetables . . . because their solid fibres consist principally of carbon, and their fluids of water . . . When vegetable leaves are exposed to the sun's light they seem to give up oxygen gas. . . . Sugar is the principal nutriment of both animal and vegetable beings . . . the wonderful effect of vegetable digestion in producing sugar may be deduced from the great product of the sugar cane"[49].

Although Ingenhousz (1779) had shown that plants absorb carbon dioxide and give out oxygen in sunlight, Darwin goes much further towards the complete reaction, which was elucidated by Liebig and others some 40 years later.

Darwin also discusses other elements essential to plant nutrition, and was the first to recognise the importance of nitrogen and phosphorus[50]. He discusses nitrogen in detail, correctly specifying the role of ammonia and the formation of nitrates. He was well on the road towards defining the carbon and nitrogen cycles. Phosphorus he believed to be present in all plants because of their tendency to phosphoresce when decaying, and he recommends a search for phosphates as fertilisers—particularly calcium phosphate, since he also thought calcium was a vital element.



nothing is to be overcome but the vis inertiae & friction, as the water box is always the same weight, whether the boat is in it or not, since the boat whether full or empty will detrude just as much water as its own weight.

Perhaps levers like fire engine beams, would be preferable to wheels? perhaps the best ballance would be another trough full of water, as they might both of them be let dry if necessary occasionally.

Fig. 2 Some of Darwin's sketches for a canal lift, from his Commonplace Book (1777). Reproduced by courtesy of The Royal College of Surgeons of England.

Among the many other bright ideas in *Phytologia* are sewage farms, biological control of insects, engineering drawings for new water pumps and drill ploughs, a seminal discussion of the individuality of buds, and proposals for a system of artesian wells[51].

The pageant of life

The last of Darwin's five books is another long poem, *The Temple of Nature*, published posthumously in 1803. This is in my view his finest achievement, and its republication in facsimile by Scolar Press recently[52] should bring it back into circulation after 150 years of unjust neglect. In *The Temple of Nature* Darwin traces the evolution of life from microscopic specks in primaeval oceans through plant life, fishes, amphibia, land animals and birds:

> Organic Life beneath the shoreless waves
> Was born, and nurs'd in Ocean's pearly caves;
> First forms minute, unseen by spheric glass,
> Move on the mud, or pierce the watery mass;
> These, as successive generations bloom,
> New powers acquire, and larger limbs assume;
> Whence countless groups of vegetation spring,
> And breathing realms of fin, and feet, and wing[53].

The couplets are just as bright as in *The Botanic Garden* and Darwin manages to cram in even more information per line. But the really astonishing feature is the calm and correct description of the processes of evolution more than 50 years before the *Origin of Species* and 100 years before the details were generally agreed. For example, Darwin nonchalantly tells us about the transformation from fish through amphibia to reptiles. Some of the marine life, he says,

> Leaves the cold caverns of the deep, and creeps
> On shelving shores, or climbs on rocky steeps.
> As in dry air the sea-born stranger roves,
> Each muscle quickens, and each sense improves;
> Cold gills aquatic form respiring lungs,
> And sounds aerial flow from slimy tongues[53].

The pressures that shape evolution are expressed with equal verve as he describes the struggle for existence among animals, insects, fish and, here, plants:

> Yes! smiling Flora drives her armèd car
> Through the thick ranks of vegetable war;
> Herb, shrub, and tree, with strong emotions rise
> For light and air, and battle in the skies;
> Whose roots diverging with opposing toil
> Contend below for moisture and for soil[54].

"Where shall we find a benevolent idea to console us" amid so much slaughter, he asks. The answer is provided by his idea of organic happiness: he reminds us that

> when a Monarch or a mushroom dies,
> Awhile extinct the organic matter lies;
> but it soon teems with microscopic life again—
> Reproduction strives
> With vanquish'd Death—and Happiness survives.

Invention and research

Concentrating on Darwin's books, as I have done, brings out the variety of his accomplishments, but leaves unrecorded his contributions to technology and science. His Lunar Society friends looked on Darwin as primarily an inventor, and Boulton made a contract to pay him £1,000 for a developed version of his speaking-machine, which could pronounce many words "with so great nicety as to deceive all who heard it unseen". He constructed a mechanical "bigrapher", an early office copying machine, which worked well and inspired Watt to invent a chemical copying method "which beats your bigrapher hollow". He designed a number of water machines, including some elaborate pumps, an automated water closet, a wire-drawn ferry and a canal lift (Fig. 2), identical in design with those brought into operation 100 years later. Other paper designs include an artificial bird with flapping wings powered by a compressed-air bottle (Fig. 3), a sketch of a hydrogen-oxygen rocket motor, and telescopes made of numerous small lenses or mirrors: all three of these have proved prophetic[55].

Darwin did not devote much time, except in his medical work, to what we would call scientific research, but he still managed to achieve more than most scientists living today can hope for. His first two papers in the *Philosophical Transactions* are of little interest now, but they secured him election as a Fellow of the Royal Society in 1761, the first of five generations of Darwins to become Fellows. In one of his later papers, in 1785, he offers the first proper explanation of the principle of the artesian well[56]. But it is in his last paper in the *Philosophical Transactions*, in 1788, that he makes his most important contributions to physical science. In this paper[57] he gives the first clear statement of the principle of adiabatic expansion[58], fundamental to all heat engines: his discovery sprang from long familiarity with steam engines and Watt's problems in improving them. Darwin then applied the principle to explain the formation of clouds: "when large districts of air are raised . . . they become so much expanded by the great diminution of pressure over them . . . that . . . the air by its expansion produces cold and devaporates"[57]. Meteorology was a lifelong interest with him, and in addition to this discovery of the main mode of cloud formation he also recognised the existence of cold and warm fronts and of "rolling cylinders of air" in the lee of mountains[10].

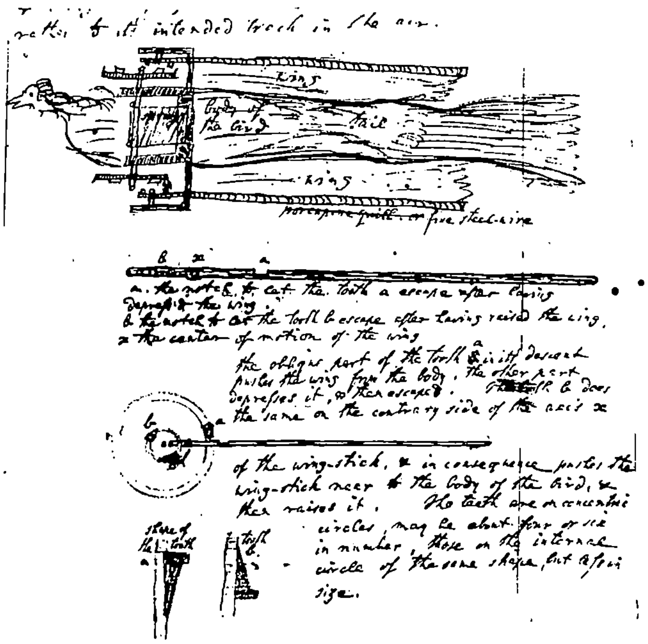

its intended track in the air.
wing
body of the bird
tail
wing
porcupine quill or fine steel-wire
b
x
a
a. the notch to cut the tooth a escape after having depressed the wing.
b the notch to cut the tooth b escape after having raised the wing.
x the center of motion of the wing
the oblique part of the tooth a in its descent pushes the wing from the body, the other part depresses it, & then escapes. The tooth b does the same on the contrary side of the axis x
of the wing-stick, & in consequence pushes the wing-stick near to the body of the bird, & then raises it. The teeth are on concentric circles, may be about four or six in number, those on the internal circle of the same shape, but less in size.
shape of the tooth a
tooth b.
b
a

Fig. 3 Darwin's artificial bird (1777). In this version the model is energised by a wound spring, but Darwin suggests a compressed-air bottle as an alternative source of power. Reproduced by courtesy of The Royal College of Surgeons of England.

In this article I have mentioned thirty-six subjects to which Darwin made pioneering contributions. I have left unmentioned thirty-nine others, including abolition of slavery, aesthetics, afforestation, animal camouflage, artificial insemination and aurorae—to quote only those beginning with 'a'. Of Darwin we can truly say, "We shall not look upon his like again". Yet schoolchildren and undergraduates in English-speaking countries complete their 'education' without hearing anything of Erasmus Darwin—a paradox for pedagogues and publishers to ponder. And, now that Darwin's poems are in print again, librarians have it in their power to popularise him.

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Noradrenaline binding and the search for catecholamine receptors

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The binding of ^{3}H-noradrenaline to intact cells and microsomes seems not to measure catecholamine receptor interactions, but rather a membrane catechol-binding protein which may be related to the enzyme, catechol-O-methyl transferase. True receptors are probably scarce and detection will require catecholamine derivatives of much higher specific activity than are at present available.

In the past few years considerable progress has been made in the identification of membrane-bound receptor structures for polypeptide hormones (insulin, glucagon, ACTH) in mammalian tissues and for cholinergic drugs in specialised tissues of certain fish. These studies have been based on the selective binding of highly radioactive (about 1 Ci μmol^{-1}) hormone derivatives to isolated cells or membrane preparations. These interactions demonstrate very strict specificity, saturability indicative of a finite and limited number of receptors, reversibility, and affinity and kinetic rate constants of binding which correlate with the biological properties of the hormone. Although radioactively labelled catecholamines have been used successfully for many years in the elucidation of important transport, storage and binding functions of adrenergic neurones[1–10], only recently have these labelled compounds been used to measure putative β-adrenergic receptors. Interactions between ^{3}H-labelled catecholamines and receptors have been described for isolated liver[11–13] and heart[14–17] microsomal preparations, spleen capsule[18], cultured myocardial cells[19], and turkey erythrocyte ghosts[20,21]. Here we describe our attempts to identify β-adrenergic receptors in intact cells and in microsomes from various tissues. Our data indicate that the binding of ^{3}H-noradrenaline does not measure catecholamine receptor interactions but rather a membrane catechol-binding protein which may be related to the enzyme, catechol-O-methyl transferase (COMT).

The binding of ^{3}H-(−)-noradrenaline to microsomal preparations (see legend to Table 1) from liver, heart and fat cells is a saturable process with respect to the hormone, although the binding is complicated by the existence of at least two separate binding processes and by an apparent cooperativity at low hormone concentrations. In microsomes stored at −20° C for 1 month the first plateau of binding indicates a capacity of about 1.4 nmol of hormone per mg of membrane protein in liver, 1.1 nmol in fat cell and 1.7 nmol in heart microsomes. These binding capacities are 10^3 to 10^4 times larger than for insulin[23,25] and glucagon[27,28] binding to fat and liver cell membranes. Using intact isolated fat cells, which are especially useful because of the homogenous nature of the cell type, absence of neural tissue and exquisite sensitivity to catecholamines, the data indicate a maximal ^{3}H-(−)-noradrenaline binding capacity of about 5×10^6 molecules per cell, which is at least 500 times greater than the binding capacity of these cells for insulin[22]. Myocardial cells in culture bind about 2.8×10^6 molecules per cell[19]. The concentration of unlabelled noradrenaline required to decrease the binding by 50% in fat, liver and heart cell microsomes and in intact fat cells is about 10^{-6} M.

These data must be interpreted very cautiously because the binding capacity of microsomes (in liver, heart and fat cells) increases strikingly during storage. Storage of fresh liver microsomes at −20° C in 50 mM sodium phosphate buffer resulted in a seven-fold increase in binding after 8 d and a fifteen-fold increase after 17 d; little further change occurs after 30 d. Storage of fresh liver microsomes at 4° C in 0.1 M Tris-HCl, *p*H 8.6, for 16 h results in a nine-fold increase in binding. Binding to stored fat cell microsomes, relative to insulin binding, is at least 200 times greater in the microsomes than in the intact cells.

Non-radioactive (+)- and (−)-noradrenaline compete in an identical way with ^{3}H-(−)-noradrenaline for binding to intact fat cells. The apparent affinity constants estimated from such inhibition studies are about 1 to 3×10^{-6} M. The apparent affinity constant calculated from dose-response studies of lipolysis (see legend to Table 1) is about 2×10^{-7} M. If sodium metabisulphite (20 μg ml^{-1}) is added to the medium to prevent oxidation of the catecholamine during the incubation (60 min, 37° C), the lipolytic dose response curves become hyperbolic and a half-maximal response was obtained at 6.5×10^{-8} M. Thus, there are substantial differences (about thirty-fold) between the apparent affinities for binding and for activation of a biological response in the identical, intact cell system.

The complete lack of stereospecificity for binding of ^{3}H-(−)-noradrenaline has been repeatedly confirmed in numerous systems including microsomal preparations from rat liver, heart, spleen and fat cells, sheep spleen capsule and pineal gland, and rabbit superior cervical ganglion. Even small degrees of stereospecificity are not detectable despite numerous variations in the concentration of ^{3}H-(−)-noradrenaline, temperature and time of incubation, rapidity of preparation of tissue, and composition of the buffer and cations. Others have also reported that the binding of catecholamines to liver[13] and heart[17] microsomes and turkey erythrocyte ghosts[21] is not stereospecific.

Specificity for catechols

Without exception, all compounds having a 3,4-dihydroxyphenolic moiety can effectively inhibit the binding of ^{3}H-(−)-

noradrenaline to microsomal preparations (Table 1). No reproducible differences were detectable in the relative ability of a widely divergent group of catechols to compete for binding. Pyrocatechol and dihydroxymandelic acid, which are not catecholamines, can displace binding effectively and completely. The presence of a third hydroxyl group at position 6 of the catechol ring (6-hydroxydopamine) resulted in an eight-fold lower apparent affinity. Compounds related to biogenic amines but lacking a catechol group are ineffective. Very similar results were obtained with microsomes from fat cells, liver and heart. The specificity of binding is thus restricted to catechol derivatives. This requirement for the catechol structure has also been reported for liver[12,13] and heart[17] microsomes and for turkey erythrocyte ghosts[21].

(+)-Noradrenaline, which can completely block the binding of ^{3}H-(−)-noradrenaline as effectively as (−)-noradrenaline, was ineffective in eliciting lipolytic responses in fat cells at concentrations (5 μg ml^{-1}) more than 1,000 times greater than those of (−)-noradrenaline which cause detectable lipolysis (Table 2). Furthermore, the (+)-isomer did not modify at all the lipolytic response to (−)-noradrenaline, even when its concentration was 1,000 times greater. Other catechols which effectively compete for binding, which possess virtually no intrinsic β-adrenergic activity in fat cells, and which do not modify the activity of (−)-noradrenaline or (−)-isoproterenol include dopamine, pyrocatechol (Table 2) and 3,4-dihydroxymandelic acid.

Table 1 Competition by Various Compounds with Binding of ^{3}H-(—)-Noradrenaline to Liver Cell Membranes

Addition	Concentration (μg ml^{-1})	Specific c.p.m. bound
None		17,700±500
(−)-Noradrenaline	0.5	9,200±200
	1.5	4,900±100
(+)-Noradrenaline	0.5	9,000±300
(−)-Isoproterenol	0.5	8,600±400
	1.5	4,800±300
Dopamine	0.5	6,900±500
	1.5	4,100±200
α-Methylnoradrenaline	0.5	8,800±300
3,4-Dihydroxymandelic acid	0.5	7,500±400
	1.5	4,600±300
Pyrocatechol	0.5	8,500±200
	1.5	4,900±300
6-Hydroxydopamine	1.5	14,100±200
	5.0	8,600±300
	10.0	5,100±100
(−)-Phenylephrine	50	16,800±400
Tyramine	50	17,500±500
Propranolol	500	14,200±500
Metanephrine	50	17,200±300
Phentolamine	50	16,900±500
Phenylethylamine	50	17,400±400

Triplicate samples (0.25 ml) containing 0.3 mg of protein per ml of Krebs-Ringer-bicarbonate (KRB) buffer (*p*H 7.4) were preincubated (24° C) for 3 min with the indicated compound, 0.2 μM ^{3}H-(−)-noradrenaline (2.6×10^5 c.p.m., 6.4 Ci mmol^{-1}) was added and incubations were continued for 30 min at 24° C. Binding was determined by Millipore membrane (cellulose acetate) filtration, which allows the samples to be cooled (4° C), filtered and washed (4° C) within 10 s, thus decreasing the probability of dissociation of the complex[22,23]. For all samples controls were performed in which unlabelled (−)-noradrenaline (50 μg ml^{-1}) was added before ^{3}H-(−)-noradrenaline; this binding was considered non-specific, and was subtracted to calculate specific binding[22,23]. Non-specific binding is generally less than 0.5% of the radioactivity in the medium and less than 10% of the total bound except in fat cells and turkey erythrocytes and ghosts where at high (10^{-6} M) hormone concentrations a larger part may be non-specific. Liver membranes (Sprague-Dawley rats) are the microsomal pellet of homogenates prepared in 0.25 M sucrose[24,25]. Fat cell membranes represent the total particulate fraction of intact fat cells homogenised in Krebs-Ringer-bicarbonate buffer[26]. Rat heart microsomes were prepared from homogenates (Polytron, 3 min, 0.25 M sucrose) by the centrifugation procedures described[14,17], which result in very low yields of material, as well as by collecting the 2,500*g* (15 min) pellet after discarding the material, sedimenting at 300*g* (15 min); the results were similar with both preparations. Intact turkey erythrocytes were used after washing thoroughly with ice-cold 50 mM sodium phosphate, *p*H 7.4, and suspending in KRB buffer, *p*H 7.0. Ghosts were prepared each day by freezing and thawing followed by washing as described above; the ghosts are devoid of haemoglobin and most are nucleated. Glass fibre paper filters (Whatman, GF/C 2.4 cm) were used in the filtration assay of erythrocytes and ghosts since the high concentrations (1 to 8×10^8 cells ml^{-1}) required to detect binding caused plugging of Millipore filters. All binding studies were performed in KRB buffer (*p*H 7.4), although generally similar findings were obtained with phosphate buffers.

Table 2 Lipolytic Activity of Catechol Substances, and their Effect on the Lipolytic Activity of (—)-Noradrenaline in Isolated Fat Cells

Addition	Concentration (ng ml^{-1})	Glycerol released
None		13.6
(−)-Noradrenaline	10	48.7
	50	71.0
(−)-Isoproterenol	0.5	27.4
(+)-Noradrenaline	500	14.4
	5,000	14.6
Pyrocatechol	2,000	14.5
Dopamine	500	16.2
3,4-Dihydroxymandelic acid	500	15.1
(+)-Noradrenaline+	500	
(−)-Noradrenaline	10	51.4
(−)-Isoproterenol	0.5	28.2
Pyrocatechol+	2,000	
(−)-Noradrenaline	10	49.8
Dopamine+	500	
(−)-Noradrenaline	10	52.9
3,4-Dihydroxymandelic acid+	500	
(−)-Noradrenaline	10	50.1

Fat cells were incubated for 80 min in KRB buffer–3% albumin, 7 μg per ml of sodium metabisulphite, and the indicated compound. Lipolysis was determined by measuring the release of glycerol in the medium[30,31]. Results are expressed as μmol of glycerol per mmol of cell triglyceride. The lipolytic potency of (−)-phenylephrine, pronethalol, phentolamine, and 6-hydroxydopamine was at least 100 times lower than that of (−)-noradrenaline while the potency of (−)-isoproterenol was five to ten times greater.

(+)-Noradrenaline did not stimulate the activity of adenylate cyclase in microsomes of isolated fat cells except at concentrations 200 to 1,000 times (depending on the batch) greater than those of (−)-noradrenaline (Fig. 1). This probably resulted from trace contamination with the (−)-isomer. The (+)-isomer in ten- to fifty-fold molar excess did not inhibit the activity stimulated by (−)-noradrenaline (Fig. 1). The catechol compounds, dihydroxymandelic acid, α-methyl dopa and pyrocatechol did not stimulate adenylate cyclase activity at concentrations as high as 10^{-4} M, and they did not inhibit (at 10^{-4} M) the stimulation (5.5-fold) caused by 5×10^{-6} M (−)-noradrenaline.

In liver microsomes maximal stimulation of adenylate cyclase (conditions as in Fig. 1) occurred with 10^{-5} M (−)-noradrenaline. Concentrations higher than 0.1 mM reversed the stimulation, presumably non-specifically. (+)-Noradrenaline did not inhibit the (−)-isomer in liver, even at 100-fold higher concentrations, provided its concentration did not exceed 1 mM. Such high (+)-noradrenaline concentrations were non-specifically toxic since they also inhibited activities stimulated by glucagon and fluoride. Fluoride (10 mM) stimulation was inhibited about 18% and 60%, and glucagon (10^{-6} M) was inhibited 20% and 100%, by 1 mM and 10 mM, respectively, of (+)-noradrenaline.

The non-catechol, *m*-methanesulphonamide derivative of isoproterenol, Soterenol (compound number 49)[32,33], stimulated lipolysis in fat cells and adenylate cyclase activity in fat cell membranes, and its activity was blocked by propranolol. Its apparent affinity was similar to that of (−)-noradrenaline. Despite this, very high concentrations (0.5 mM) of Soterenol (gift from Dr W. T. Comer, Mead Johnson) only decreased

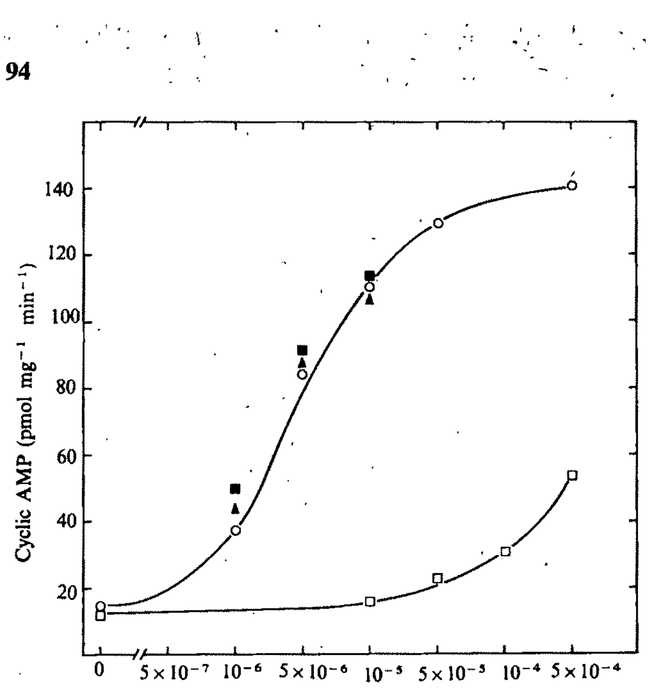

Fig. 1 Effect of (+)-noradrenaline on (−)-noradrenaline stimulation of adenylate cyclase activity in isolated fat cell microsomes. The incubation mixture (0.1 ml) contained 25 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$, 0.1 mM $MnCl_2$, 0.1 mM GTP, 2.5 mM aminophylline, 0.1% (w/v) albumin, 0.1 mM α-^{32}P-ATP (6×10^6 c.p.m.) and 100–200 μg of membrane protein; 5 mM phosphoenolpyruvate and 60 μg ml^{-1} of pyruvate kinase were used to regenerate ATP. After 5 min at 37° C, the tubes were placed in boiling water for 2 min. A recovery mixture (1.0 ml) containing ^{3}H-cyclic AMP was added to each sample. Cyclic AMP was isolated on a column containing 1 g of alumina that was eluted with 2.0 ml of 25 mM Tris-HCl (pH 7.6). Values are averages of triplicate replications. □, (+)-Noradrenaline; ○, (−)-noradrenaline; ▲, (−)-noradrenaline with 10^{-5} M (+)-noradrenaline; ■, (−)-noradrenaline with 5×10^{-5} M (+)-noradrenaline.

the binding of ^{3}H-(−)-noradrenaline (0.2 μM) by only 13% in fat cells, 14% in liver, and 22% in heart microsomes.

By two different methods the ^{3}H-(−)-noradrenaline which binds to liver, heart and fat cell membranes could not dissociate over a 40- to 80-min period at 4° C, 24° C, or 37° C (Fig. 2). The results were similar if the hormone-membrane complex was incubated for only 10 min at 24° C or 4° C before dissociation was studied, and this lack of dissociation was observed with fresh or stored microsomes. These results contrast with the immediacy of reversal of the catecholamine effect observed upon removal of the hormone from the medium in a variety of biological systems, including fat cells. HCl (0.4 N) did not reverse but instead slightly enhanced the complex between noradrenaline and liver or heart microsomes (Fig. 2, insert). Guanidine-HCl (5 M) increased, and 0.4 N NaOH depressed, the binding. The essentially irreversible nature of the binding and the facile oxidation of catechols have hindered chemical identification of the membrane-bound ^{3}H.

Relation with enzyme COMT

In agreement with others[13,17], proteolytic (Pronase) digestion of membranes destroyed subsequent binding activity, indicating that protein components are involved. The specificity of binding described above is very different from that observed for the transport, storage and binding functions of adrenergic neurones or storage vesicles[1–10]; furthermore, isolated fat cells and erythrocytes are devoid of such structures.

The strict requirement of the binding process on the presence of a catechol function suggests a possible relationship with the enzyme, catechol-O-methyl transferase (COMT). This enzyme has similar affinities for a wide variety of catechols, even for those lacking a ring side chain, and it does not distinguish between (+)- and (−)-forms of noradrenaline[34–37]. Although COMT is present in the soluble fraction of most tissues, its presence in microsomes is well established[38–40]. In isolated fat cells the enzyme is found predominantly in a membrane-bound form[40]. Although the particulate and soluble enzymes differ in certain respects[38], both demonstrate broad substrate specificity[38,39].

The presence of COMT activity[34,35] in the liver, fat and heart microsomal preparations used in these studies was confirmed using ^{3}H-(−)-noradrenaline or ^{14}C-S-adenosylmethionine[41] as substrates. As observed for COMT[13,17], reagents that blocked sulphydryl groups (such as p-chloromercuribenzoate) also blocked ^{3}H-(−)-noradrenaline binding. Also like COMT, (−)-noradrenaline binding required divalent cations; binding decreased 50% with 2 mM EDTA in liver membranes. Some well known[37,42,43] inhibitors (tropolone, pyrogallol, quercetin and U-0521) of COMT which do not have β-adrenergic activity could also inhibit the binding to liver and heart microsomes (Table 3).

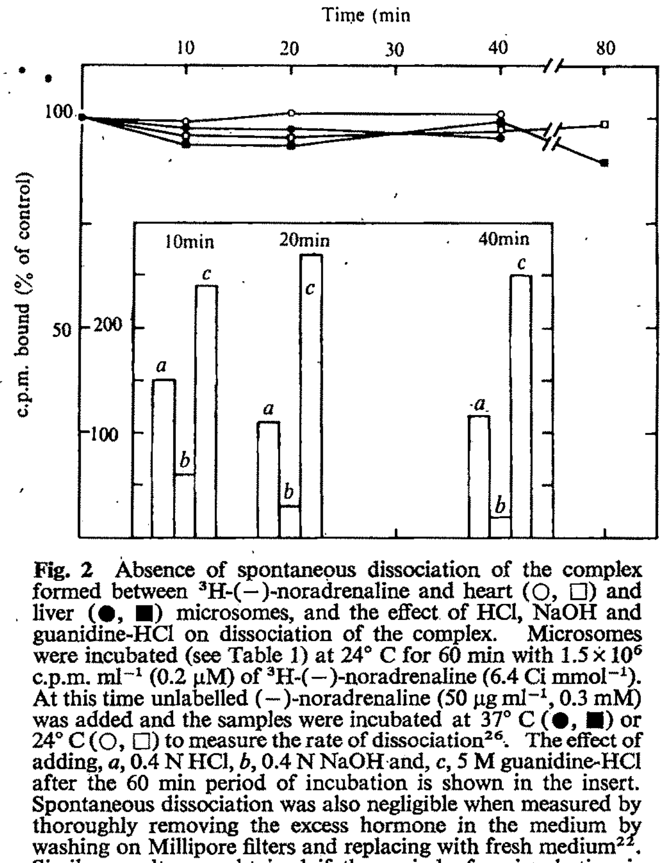

Fig. 2 Absence of spontaneous dissociation of the complex formed between ^{3}H-(−)-noradrenaline and heart (○, □) and liver (●, ■) microsomes, and the effect of HCl, NaOH and guanidine-HCl on dissociation of the complex. Microsomes were incubated (see Table 1) at 24° C for 60 min with 1.5×10^6 c.p.m. ml^{-1} (0.2 μM) of ^{3}H-(−)-noradrenaline (6.4 Ci $mmol^{-1}$). At this time unlabelled (−)-noradrenaline (50 μg ml^{-1}, 0.3 mM) was added and the samples were incubated at 37° C (●, ■) or 24° C (○, □) to measure the rate of dissociation[26]. The effect of adding, *a*, 0.4 N HCl, *b*, 0.4 N NaOH and, *c*, 5 M guanidine-HCl after the 60 min period of incubation is shown in the insert. Spontaneous dissociation was also negligible when measured by thoroughly removing the excess hormone in the medium by washing on Millipore filters and replacing with fresh medium[22]. Similar results are obtained if the period of preincubation is short (10 min), or if the temperature of preincubation is 4° C.

S-Adenosyl-L-methionine (SAM), a compulsory substrate for COMT activity[34,35,43], markedly increased (two- to four-fold at 1 mM) the rate of binding of ^{3}H-(−)-noradrenaline to liver and heart microsomes (Fig. 3); effects were detectable at concentrations below 0.1 mM. The effect of SAM was most pronounced on freshly prepared microsomes, and little effect could be observed in preparations stored frozen for more than 3 weeks. SAM did not dissociate bound radioactivity even if added after removal of the free ^{3}H-(−)-noradrenaline in the medium. Thus, SAM did not simply increase the rate of turnover of the catecholamine-membrane complex. The normal product of SAM hydrolysis by COMT, S-adenosylhomocysteine (SAH), inhibited weakly the binding to heart and liver microsomes; 10% to 20% inhibition (measured at 10 min, 24° C) occurred in the presence or absence of SAM (1 mM) with 5 mM SAH. The normal (−)-noradrenaline

Table 3 Effect of Inhibitors of Catechol-O-Methyl Transferase on Binding of ^{3}H-(—)-Noradrenaline to Liver Membranes

Addition	Concentration (μg ml^{-1})	Specific ^{3}H-noradrenaline bound (c.p.m.)
None		8,200 ± 300
(−)-Noradrenaline	2.5	3,300 ± 100
	0.5	5,400 ± 300
	0.1	7,600 ± 200
Tropolone	0.5	5,900 ± 100
	0.1	6,800 ± 200
U-0521	10	6,300 ± 100
	0.5	7,300 ± 300
Pyrogallol	10	3,600 ± 100
	0.5	6,100 ± 200
Quercetin	10	3,100 ± 200
	0.5	6,100 ± 300

Membranes (0.2 ml containing 1.1 mg of protein per ml) were incubated (24° C) for 3 min with the indicated compound followed by 60 min at 4° C with 0.9 μM ^{3}H-(−)-noradrenaline (4.8×10^5 c.p.m., 6.4 Ci mmol^{-1}) as described in Table 1. Similar results were obtained by incubating for 30 min at 24° C, and with heart microsomes. U-0521 is 3,4-dimethoxy-5-hydroxybenzoic acid[37].

product of transferase activity, (−)-normetanephrine, did not alter the binding (including rates) of ^{3}H-(−)-noradrenaline (0.1 μM) in the absence or presence of SAM even when tested at concentrations as high as 0.3 mM. The material bound to membranes was probably not a simple product of COMT catalysis.

The close analogue of SAM, S-adenosylethionine (SAE), profoundly decreased the binding of ^{3}H-(−)-noradrenaline to heart and liver microsomes (Table 4). Significant effects occurred with 5 μM SAE. SAE could decrease the binding even more effectively than nearly saturating concentrations (5 μg ml^{-1}) of unlabelled (−)-noradrenaline. Inhibition was observed only if SAE is added before ^{3}H-(−)-noradrenaline, and no dissociation was seen if it was added after the complex has incubated for 10 to 30 min at 24° C or 10 to 60 min at 4° C, even if the microsomes were washed before testing. SAE abolished the effects of SAM described above, and Dixon plots suggested competitive kinetics of inhibition with an apparent dissociation constant of about 80 μM (heart microsomes). SAE is a potent inhibitor of liver and heart microsomal COMT activity. When assayed with 30 μM ^{14}C SAM and 1 mM 3,4-dihydroxybenzoic acid (under conditions giving zero order kinetics), concentrations of SAE of 0.3 mM, 30 μM, and 3 μM inhibited COMT activity of liver microsomes by 85%, 56% and 15%, respectively.

Table 4 Inhibition of Binding of ^{3}H-(—)-Noradrenaline to Heart and Liver Membranes by S-Adenosylethionine

Addition	Total ^{3}H-noradrenaline bound (c.p.m.) Liver microsomes	Heart microsomes
None	13,500	7,200
(—)-Noradrenaline, 5 μg/ml	800	600
S-Adenosylethionine, 5 mM	500	400
1 mM	850	1,000
0.2 mM	1,250	3,800
40 μM	5,900	4,900
5 μM	9,300	6,700

The experiments were performed as described in Fig. 3. Incubations (24° C, 40 min) (0.2 ml) contained 0.24 mg (liver) or 0.30 mg (heart) microsomal protein and 1.2×10^5 c.p.m. (liver) or 2.0×10^5 c.p.m. (heart) of ^{3}H-(−)-noradrenaline (6.4 Ci/mmol).

3-Mercaptotyramine, which selectively and irreversibly inactivates soluble COMT presumably by forming disulphide complexes at the active site of the enzyme[45], is a very potent inhibitor of noradrenaline binding to liver and heart microsomes. Incubation of heart microsomes for 15 min (37° C) with 0.3 mM, 30 μM, 6 μM, 1 μM and 0.3 μM 3-mercaptotyramine (a gift from Dr C. R. Creveling) decreased the subsequent binding (30 min, 24° C) of ^{3}H-noradrenaline (0.8 μM) by 100%, 91%, 73%, 28%, and 15%, respectively.

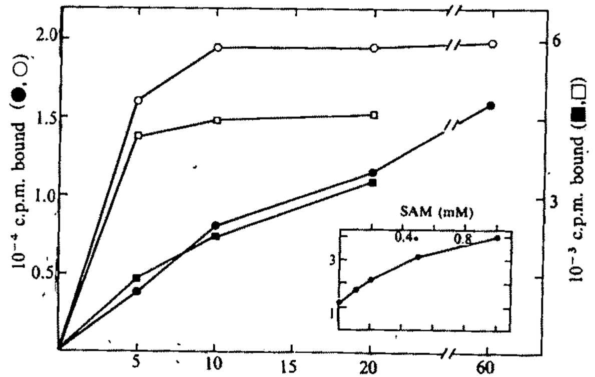

Fig. 3 Effect of S-adenosylmethionine (SAM) (○, □) on the binding of ^{3}H-(−)-noradrenaline (4.5×10^5 c.p.m. for heart, 1.9×10^5 c.p.m. for liver; 6.4 Ci mmol^{-1}) to liver (●, ○) and heart (■, □) microsomes. SAM was added immediately before the radioactivity. The protein content was 0.24 (heart) or 0.21 (liver) mg per 0.2 ml, and incubations were at 24° C. Insert shows the effect of SAM concentration on heart microsomes measured at 5 min.

Since noradrenaline binding is essentially irreversible, and since SAM does not release the bound material, the binding may represent an aberrant, covalent enzyme-substrate intermediate which is not susceptible to completion by the normal catalytic process. The very large increase in binding capacity during storage of microsomes or by treatment with Tris-HCl may reflect such an alteration of the enzyme. It may be relevant that dithiothreitol (DTT) potently inhibits the binding to liver and heart microsomes. Binding (30 min, 24° C) of 0.4 μM ^{3}H-(−)-noradrenaline to heart microsomes is decreased by 82%, 73%, 48% and 15% by 1 mM, 0.25 mM, 60 μM and 15 μM, respectively, of DTT. Although the activity of soluble COMT (heart or liver) is not inhibited by 3 mM DTT, the activity of microsomal, particulate preparations is enhanced four- to six-fold by concentrations of DTT as low as 0.6 mM. DTT thus may revert an altered particulate enzyme into a form that no longer binds catechol substrates irreversibly.

Solubilisation of catechol binding protein

As described by others[13,15,16], the binding protein can be solubilised from microsomes with non-ionic detergents. We studied extensively the material solubilised from fat and liver microsomes with Triton X-100. The soluble protein-^{3}H-noradrenaline complex would be assayed by precipitation with 65% $(NH_4)_2SO_4$, followed by Millipore filtration as described for microsomes, or by gel filtration on Sephadex G-25. As in microsomes, binding was destroyed by Pronase, was saturable with two distinct plateaus (in liver, K_d values were about 3×10^{-7} M and 2×10^{-5} M), was suppressed by unlabelled hormone, was lacking in stereospecificity, demonstrated specificity only for catechols, and could not be dissociated even during 2 h at 37° C. Binding was inhibited by SAE (50 μM inhibited the binding of 0.3 μM ^{3}H-noradrenaline to liver extracts by 75%), and the rate of binding was increased by SAM. 3-Mercaptotyramine[45] inhibited binding profoundly; 6 and 1 μM (15 min, 37° C) inhibited subsequent binding (liver) by 97% and 84%, respectively. Binding was also very sensitive to DTT. A most unusual property was the ability of 5 M guanidine-HCl and 2% sodium dodecyl sulphate to increase binding by 50% and by two- to five-fold, respectively; unlabelled hormone (10 µg per ml) prevented this increased binding, which required tissue extract, and ^{3}H-noradrenaline did not bind to the detergent or to guanidine. Binding was suppressed in 2 M NaCl and in 8.3 M guanidine-HCl, *p*H 1. The complex, once formed, did not dissociate with 2% sodium dodecyl sulphate, 0.5 N HCl, or by boiling for 4 min.

Binding to turkey erythrocytes

In accordance with the report of Bilezikian *et al.*[21], binding of ^{3}H-(−)-noradrenaline to turkey erythrocyte ghosts (see legend, Table 1) was not stereospecific and only required the catechol moiety. As in microsomes, binding was only very weakly inhibited by Soterenol, and it was inhibited by SAE and by 3-mercaptotyramine (although higher concentrations were required). Low concentrations (0.2 mM) of DTT inhibited binding, but not as profoundly as in microsomes. Unlike liver and heart microsomes, the bound ^{3}H-noradrenaline could dissociate spontaneously.

There were striking differences between the binding to intact erythrocytes and to ghosts. Freshly prepared ghosts bound thirty to seventy times more noradrenaline (per cell) than intact cells. Storage of the ghosts (−20° C, 3 d) further increased binding by a factor of two to three; binding did not change upon storing intact cells at 4° C for 3 d. An intact cell could bind a maximum of about 14,000 noradrenaline molecules, while a ghost could bind about 650,000. Intact human erythrocytes, which are unresponsive to catecholamines[46], bound about 5,000 molecules per cell.

Maximal stimulation (seven-fold) of adenylate cyclase activity (methods as in Fig. 1) in ghosts occurred with 5×10^{-5} M (−)-noradrenaline; concentrations higher than 0.5 mM reversed the stimulation, as described for microsomes. The activity of the (−)-isomer was not reversed by 100- to 500-fold higher concentrations of (+)-noradrenaline provided these did not exceed 10^{-3} M, which, as described earlier, non-specifically suppress activity. Pyrocatechol, dihydroxymandelic acid and α-methyl dopa did not stimulate the enzyme, and these compounds at 10^{-4} M and 5×10^{-4} M did not inhibit the stimulation (5-fold) of 5×10^{-6} M (−)-noradrenaline. The isoproterenol (10^{-8} M) enhanced rate of sodium outflux (15 min) in turkey erythrocytes was not modified by a 1,000-fold molar excess of DL-dopa, dopamine, vanilmandelic acid, or 3,4-dihydroxyphenylacetic acid, compounds that completely block the binding of ^{3}H-isoproterenol[46]. Although these compounds (1,000-fold excess, 0.2 mM) decreased the levels of cyclic AMP stimulated (90 min) by isoproterenol, the inhibition was only about 15%, and possible non-specific toxicity was not examined[46].

Stereospecific binding was not demonstrable in intact erythrocytes, despite the low number of binding sites compared to ghosts. The residual binding observed in the presence of pyrocatechol, 3-mercaptotyramine, SAE or DTT did not reveal stereospecificity. Despite differences in the binding between turkey erythrocytes (and ghosts) and mammalian cells or microsomes, the essential features indicate that the binding did not reflect a true β-adrenergic receptor interaction. The possible relation of binding to catechol transport has not been examined.

The binding of ^{3}H-(−)-noradrenaline to subcellular particles or cells cannot be reconciled with the view that such binding reflects β-adrenergic receptor interactions. The single most compelling argument is that certain compounds which completely prevent this binding have no intrinsic biological activity and do not measurably alter the biological activity of (−)-noradrenaline. Binding is probably related to a totally separate process which shares with catecholamine receptors only the property of recognising catechol moieties. It must be concluded that if such putative receptors exist in the materials examined, they must represent a negligible fraction of the actual binding of ^{3}H-noradrenaline observed. Estimates of the possible number of receptors in fat cells from lipolytic studies using a catecholamine, N-[2-(*p*-hydroxyphenylpropyl)]-(−)-noradrenaline (gift from Dr J. van Dijk, Philips-Duphar, The Netherlands), of very high apparent affinity (about 3×10^{-10} M), with the assumption that 10% of the molecules in the medium are bound, give a maximum figure of 10^5 sites per cell. This is five to ten times greater than the maximum number of insulin or glucagon molecules that are bound per fat cell[22]. The numbers of noradrenaline binding sites in microsomes or cells are orders of magnitude greater than the corresponding figures for peptide hormones. It may therefore not be surprising that specific receptors cannot be detected in the presence of other binding processes which exist in great excess. In turkey erythrocytes, however, the number of binding sites is very low. Assuming a cell radius of 4 µm, intact turkey cells have a binding density of about seven sites per µm^2, and ghosts about 300 sites per µm^2. (The density of insulin receptors in fat cells is 1 to 5 per µm^2, depending on cell size[22].) But despite the scarcity of interfering catechol-binding sites in erythrocytes, stereospecific binding cannot be detected.

Many attempts to detect stereospecific binding in cells and membranes after experimentally suppressing (with pyrocatechol, specific inhibitors of COMT, SAE, 3-mercaptotyramine, DTT, EDTA and treatment with *p*-chloromercuribenzoate or iodoacetamine) the catechol-binding structures have failed. In these cases the residual binding is very low. This is consistent with the probable scarcity of true receptors. Detection will probably require catecholamine derivatives of much higher specific activity than are at present available. The ^{125}I-labelled peptide hormones used to study receptors are 100 to 300 times more radioactive than ^{3}H-(−)-noradrenaline.

For technical reasons the ligand used to detect receptors should have the highest possible affinity. It can be calculated from $K_d = k_1/k_{-1}$ (K_d, dissociation constant; k_1 and k_{-1}, rates of association and dissociation, respectively) that a complex having a k_1 of 5×10^5 mol^{-1} s^{-1}, which is a very low estimate (ten times less than that of insulin receptors, 10^4- to 10^5-fold less than diffusion encounters), and a K_d of 5×10^{-8} M would exhibit a half-life of about 30 s. Since the true figure may well be ten times lower, it is possible that extensive dissociation of the complex can occur during washing of the particles or cells in the binding assay, especially if it is not performed at low temperatures.

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Typographical errors and technological solutions

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A recently reported typographical error in our global computer simulation model did exist, but had only a small quantitative effect on the published results. The error was not responsible for the pollution crisis mode; its removal does not stabilise the model system and the conclusions are unaffected by the numerical change.

In a recent issue of *Nature*[1], Boyle reports finding a typographical error in the computer simulation model used as a background for our book, *The Limits to Growth*[2]. Boyle states that the error is responsible for the pollution crisis mode of the model and concludes that "affluence without restrictive social policies is apparently attainable". For the benefit of the many research groups currently working with our technical report on the model[3], as well as the general public interested in the policy questions raised by *The Limits to Growth*, we would like to clarify the nature of this error and its effect on our conclusions.

Error

Tabular functions are often used in system dynamics models to express the nonlinear relationship of one variable to another. In early versions of the world model we employed such a table function to represent the relative amount of pollution released as a function of the industrial output per capita. The table, called Pollution Generation Multiplier from Output Table (PGMOT), contained one mistyped value as indicated below (the values in the table express pollution relative to total global emissions in 1970, and they correspond to values of industrial output per capita over the range from $0 to $1,600 in steps of $200).

```
T PGMOT=0/0.87/1.64/2.5/3.5/4.5/5.5/6.5/7.5
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The mistyped number corresponds to a value of industrial output per capita of $1,000 (equivalent to a GNP per capita of about $4,000) and will cause an error in the model output whenever the value of industrial output per capita lies between $800 and $1,200. That situation occurs in three of the twelve model runs shown in *The Limits to Growth*, Figs 40, 41 and 42. In the other nine runs, industrial output per capita remains below the region where the PGMOT function is in error, and the error has no effect.

The typing error in PGMOT was corrected in our laboratory before the first draft of the technical report was completed in April 1972. To our knowledge, no research group working with any draft of the technical report has been given a program containing the error. We were under the impression that the figures in *The Limits to Growth* were drawn from the corrected version of the program, and we are indebted to Boyle for pointing out our mistake. The error, however, has no effect on the general message of the book and it does not alter our conclusions in any way.

That statement may be better understood if we examine the error in the full context of the model assumptions.

Implications

The basic assumptions of the world model are:

(1) Population and capital by their very nature tend to grow exponentially; the amount added to either population or capital each year is partially determined by the amount of population or capital already present. This exponential tendency is enhanced by current short-term, pro-growth value systems.

(2) There are limits to the growth of any physical quantity on a finite Earth. The carrying capacity for human population and industry is variable; it can be expanded by technology, and it can be diminished by misuse, erosion or depletion of resources.

(3) There are significant delays in the feedback processes that act to contain population and capital within the global carrying capacity. Some of these feedback delays arise from biological or physical processes (such as population ageing and the passage of a pollutant up a food chain) and others from social processes (such as perception and communication delays and resistance to change).

These three assumptions, considerably elaborated, constitute the world model. The conclusion that follows from them is that the present global population-capital system is dynamically unstable. The combination of rapid physical growth and long feedback delays can lead to a temporary overshoot of the sustainable physical limits to population and capital. During the overshoot period the carrying capacity can be eroded by overuse, leading to an eventual decline of population and industry. The overshoot can be entirely avoided by a change of values to favour a deliberate stabilisation of population and capital. The quality of life in the stabilised state can be greatly enhanced by technologies that preserve resources and abate pollution. Technological changes alone, however, unaccompanied by value changes that lead to deliberate slowing of growth, can lead to greater overshoot by temporarily enhancing exponential growth and by masking and further delaying the feedback pressures from physical limits.

This general conclusion is the most significant one to be drawn from the model or from *The Limits to Growth*. The model provides no information about whether the value changes that lead to stabilisation will actually occur. No single computer run of the twelve presented in *The Limits to Growth* can be interpreted as an exact prediction of the future state of the world—in fact the precise quantitative features of the model runs were deliberately removed to discourage such interpretation. The output of such a general, aggregate model can never be, and was never claimed to be, numerically accurate. Three different models, all built around the same assumptions but incorporating different degrees of detail and different numerical parameters, have produced quantitatively different but qualitatively identical conclusions[2-4]. These qualitative conclusions about system instability and the conditions that lead to stability are the only ones upon which any policy recommendations have been based.

Although the numerical error reported by Boyle does influence the quantitative output of three computer runs, it does not influence the system instability reflected in those runs, nor does it invalidate or even address any of the basic assumptions in the model. The version of Fig. 42, illustrated in Boyle's paper, still shows a severe overshoot of industrial capital, followed by an uncontrolled collapse. Boyle states, "The pollution crisis forecast . . . has been traced to a typographical error in the program" but pollution crises occur in Figs 36 and 37 of *The Limits to Growth*, where the error is not operative, and in several figures in both *World Dynamics* (pages 10, 75, 96, 100, 105, 108–111) and the technical report[3] (chapter VII, runs 7, 8, 13, 14) where the error is not even present.

Stabilisation policies

Boyle goes beyond the question of the typographical error to suggest an alternative set of policy changes that will stabilise the model system. Many such stabilising policy sets exist, each of which leads to a quantitatively different equilibrium state. The one common characteristic of these stabilising policies is that they supply some forward-looking social pressure against growth in place of the delayed-feedback physical pressures of the natural system.

The pressures that Boyle has selected—on agricultural development, capital investment, advertising and consumer demand for food and industrial output—are not different in kind from those we used to stabilise the model. (We called our stabilising measures "deliberate constraints on growth"; Boyle prefers to call his "rational economic policies".) The major difference is that he excludes any deliberate pressure on population growth. *The Limits to Growth* model considerably underestimates the growth potential of the human population, as discussed in detail in the technical report[3] (chapter II) and so the question of whether policies that stabilise population in that model will stabilise a real population must be considered. In any event, *The Limits to Growth* was intended to raise the question of which pressures against growth will be effective and which we would prefer to live under, not to settle the question. Dr Boyle's alternative suggestion is a positive contribution to a debate that is just beginning.

We must take some issue, however, with Boyle's "real conclusion" that there is "hope for the technological solution". Even the crude representations of technology in *The Limits to Growth* model and in Boyle's paper do not lead to that conclusion. Furthermore, technology in this model is assumed to be immediate, perfectly effective, free of cost, and free of any unforeseen side effects. This lack of realism was utilised in *The Limits to Growth* only to illustrate the extreme case. In the technical report[3] (chapter VII, runs 21, 22, 23) and in detailed working papers[5] (chapters 3, 6 and 8), capital costs of technology and implementation delays are included. In these more realistic representations, reliance upon technology to bring an orderly transition to system equilibrium looks even less feasible.

Technological advance can, and we hope will, do much to make life more livable during the transition and in the stable state. We do not believe, however, that technology alone can produce a solution to problems arising from an inconsistent, short-term, anthropocentric set of values based primarily on the concept of an infinite Earth. Social problems ultimately require social solutions, not technological ones. It seems unpleasant for us to recognise the fact, but the sooner we do so, the sooner the necessary solutions are likely to be found.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Lepton Creation and the Dirac Relationship between Fundamental Constants

CAVALLO[1] has proposed an interpretation of the large number ratios between some of the fundamental constants occurring in physics and cosmology, in terms of rotating cosmological models. The purpose of this communication is to suggest another interpretation of these large dimensionless numbers in terms of lepton creation.

The starting point of this theory is the usual action principle for gravitation and electromagnetism but with the difference that charged leptons can be created or annihilated in the cosmological space-time. That is, the worldlines of these particles can have a beginning or an end, or both. The action can be written either in the field form or in the form of action at a distance[2]. The variation of the worldline of a lepton of mass m and charge e gives the following condition at the beginning or end of a worldline:

$$m\mathbf{u}_i = -eA_i \tag{1}$$

where A_i is the four-potential from the rest of the particles in the Universe and $\mathbf{u}_i$ is the four-velocity of the lepton.

I now apply this to a Robertson–Walker space-time used by most cosmologists, and used by Dirac in his discussion[3]. This represents an expanding homogeneous and isotropic Universe. In any given cosmological model of this type, it is possible in principle to calculate the A_i from charges of one species, although in practice the relevant integral may not be expressible in terms of elementary functions[4]. But if the Universe as a whole is electrically neutral the total A_i appearing on the right hand side of equation (1) will be zero.

I now propose a departure from strict neutrality in the following way. Suppose we consider particles of electric charge e. Although cosmological models differ in detail (such as deceleration parameter, the value of the λ constant, big bang or steady state, and so on), only the time-like component of A_i from all these particles is non-zero and it has the magnitude of the order of

$$Ne/R \ (R = c/H) \tag{2}$$

where N is the number of such charges in a spherical region of radius R. The parameters c and H in equation (2) are the velocity of light and the present value of the Hubble constant respectively. In the case of strict charge neutrality particles of opposite charge $-e$ will make an equal contribution $-Ne/R$ and thus cancel equation (2) exactly. But if the charge balance is a statistical effect, small fluctuations from it are likely to be present. This would mean that there is an overall electrostatic potential of the order

$$A_4 \sim \pm N^{1/2}e/R \tag{3}$$

The fluctuation effect assumed in equation (3) is of the order $N^{-1/2}(\sim 10^{-40})$.

Using equation (3) in equation (1), for a lepton created at rest in the cosmological substratum,

$$mc^2 = \mp N^{1/2}e^2/R \tag{4}$$

Since the left hand side is positive, the creation condition specifies that only the + sign on the right hand side is applicable. That is, only charge of one species can be created in a given region. We will return to this point again at a later stage, accepting for the time being the + sign in equation (4).

The relation (4) can be rewritten in a more familiar form:

$$R/(e^2/mc^2) \simeq N^{1/2} \tag{5}$$

stating that the 'radius' of the universe bears to the radius of the electron the ratio $N^{1/2} \sim 10^{40}$. This is one of the dimensionless numbers relating the fundamental constants.

The second relation follows when we take account of the relation between the Hubble's constant, the constant of gravitation G and the mean density ρ of matter in the universe. For the Friedmann model[5] with the deceleration parameter $q_0 = 1$, or for the steady state model with the C field[6],

$$\rho = 3H^2/4\pi G \tag{6}$$

Other models usually yield different coefficients of H^2/G on the right hand side, but they are of the same order as $3/4\pi$. Taking equation (6) to fix ideas, the mass of the matter in a sphere of radius R is around

$$4\pi R^3 \rho/3 \simeq c^3/HG \tag{7}$$

Observations of the Universe show that matter is largely made of equal numbers of electrons and protons at least in our neighbourhood. Taking M for the mass of a proton, the above relation gives an estimate of N:

$$NM \sim c^3/HG = c^2 R/G \tag{8}$$

If we now eliminate R between equations (5) and (8) we get

$$e^2/GMm \sim N^{1/2} \sim 10^{40} \tag{9}$$

The relations (5) and (9) are the core of the so-called Dirac relationships between fundamental constants. I now interpret these and discuss their astrophysical significance in the present picture by making the following points.

(1) Both relations express the overall dynamics of the Universe. The relation (5) arises from creation of charged leptons, the electrons and positrons in the cosmological framework. Although particles are created there is no overall loss of energy and momentum. Relation (8) may be looked upon either as an equipartition between the gravitational energy and the energy of expansion according to Einstein's equations or as a Machian relation determining the gravitational constant in terms of the matter content of the Universe[7,8].

(2) Although charge conservation is apparently violated when an electron is created, the violation is local, not global. The statistical fluctuation which requires the + sign in equation (4) for a given region is balanced by an opposite fluctuation which favours the creation of the opposite charge in another region. Thus we will tend to have electrons created in one region, positrons in another. Also the process is stable in the sense that the creation of too many electrons in a given region will produce a local contribution to A_4 which will soon cancel the cosmological contribution and thus control the process.

(3) Relation (6) owes its origin to a statistical fluctuation and hence is not exact. That is, it is not possible to fix the coefficient of $N^{1/2}$ on the right hand side which will make it exact although the coefficient will be of the order unity. The relation (8) is exact in any given cosmological model. (The

~ sign appears because I have not specified the model in my discussion.) If I use the present determinations of c, H and G, I find that $N^{1/2}\sim 7\times 10^{40}$ from equation (5) and $N^{1/2}\sim 1.3\times 10^{40}$ from equation (8)—a reasonably good agreement.

(4) It may be possible to turn equation (4) round and argue that it implies creation of leptons of different masses as H varies or as we take into account the variation in fluctuation. This raises the interesting possibility that the electron mass is not fixed all over the Universe. This avenue is worth exploring in view of the puzzles about the QSO redshifts unearthed by recent observations[9,10].

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Production of Organic Molecules by Proton Irradiation

IN recent years there has been considerable interest in the production of complex organic molecules in primitive atmospheres. The emphasis of most of this work has been on mechanisms for prebiological synthesis of amino acids and related molecules, but the feasibility of producing organic chromophores which may account for the colours observed on planets and satellites in the outer Solar System by these same processes has become a topic of research effort in the past few years.

The early experiments[1-5] employed mixtures of methane and ammonia[4,5], sometimes with hydrogen[1] or water[2,3]; and indeed amino acids (in the experiments with H_2O) and related organic molecules have been produced. More recently[6-8], in attempts to simulate atmospheres likely to contain sulphur (those of Jupiter and primitive Earth), mixtures including hydrogen sulphide have been used. The results of these experiments were qualitatively similar to the non-sulphur ones, except for the production of free sulphur, alkyl sulphides and thio-amino acids. In one case involving a sulphur-free mixture a red polymeric material was reported[4], and this is considered a tentative candidate for the red colouring agent on Jupiter.

With the exception of the work of Palm and Calvin[2], in which energetic electrons were used, one of two types of energy sources was employed: electrical discharges[1,3-5] and ultraviolet light from a mercury lamp[6-8]. Electrical discharges may be initiated by strong atmospheric turbulence (similar to the familiar electrical storms) in the primitive atmospheres, and there is a significant amount of ultraviolet flux in the solar radiation. There is another source of energy, however, that is becoming increasingly worthy of consideration: energetic protons in the solar wind and in planetary radiation belts. Considerable attention has been given to the structure and composition of the radiation belts around Jupiter and the proton (and electron) flux is believed to be far from insignificant[9]. The satellites of the outer planets certainly are exposed to the solar wind, and two of these, Io and Titan, are unusually red[10]. Titan is especially interesting as it is known to possess an atmosphere of at least H_2 and CH_4 (ref. 11). Also, protons can bombard a planetary atmosphere directly from discharges in the radiation belts. It is possible, then, for proton bombardment to have a measurable effect on the organic chemistry of primitive planetary atmospheres.

The following preliminary experiments were carried out to investigate the effectiveness of proton irradiation for producing organic chromophores. The source of the 2 MeV protons used in the experiments was a model FN tandem Van de Graaff accelerator of the physics department at Stony Brook. The beams used ranged from 870 nA (5.44×10^{12} protons cm^{-2} s^{-1} over 1/3 cm diameter) to 220 nA (1.35×10^{12} protons cm^{-2} s^{-1}). Based on the nominal model[9] for the flux in Jupiter's radiation belt at six Jupiter radii from the planet (approximately the distance of Io), these beams correspond to 1.72 and 0.43 Earth years at Jupiter per second of exposure. Thus, for typical exposures of 3 and 6 h respectively, the total proton fluences are equivalent to those of 1.9×10^{5} yr and 9.2×10^{4} yr at Jupiter.

The gas cells used were hollow aluminium cylinders, each with a gas volume of approximately 125 cm³. Attached to both ends of each cell were infrasil quartz windows, enabling visual inspection of any reaction products and gas phase spectroscopy from 0.2 to 4 µm. A very thin pure nickel foil window was attached over a hole in the centre of each cell, perpendicular to the cylinder axis, to allow entry of the proton beam into the cell without loss of gas. The cells were evacuated to less than 10^{-3} mm Hg, filled with the appropriate gas mixtures (see below) and stored in a helium atmosphere to prevent contamination by air. The exposures were accomplished by placing the cells in a large target chamber, evacuating the chamber to 5×10^{-5} mm Hg, and running the beam into the cell for the desired length of time. The beam was stopped at the rear wall of the cell with approximately 1/2 MeV of the energy lost in the nickel window and 1 MeV deposited in the gas.

Five gas mixtures were used in this first series of experiments, none of which contained water. The first cell contained 50 cm³ CH_4 and 51 cm³ NH_3 at a total pressure of 615 mm Hg, and was exposed for 3 h at 870 nA. High resolution mass spectrometry of the gas phase indicated the presence of C_2H_2, C_2H_4, C_2H_6 and their methyl homologues, as well as C_4H_2 (diacetylene), CH_3CN, and residual CH_4 and NH_3. The orange-brown oily liquid produced was analysed by computer assisted gas chromatography-mass spectrometry[12,13], which indicated the presence of the expected alkyl hydrocarbons and alkyl amines. Some unexpected and interesting products were the tricyclic alkyl amines, hexamethylene tetramine ($C_6H_{12}N_4$) and its methyl and dimethyl homologues.

The second experiment was essentially the same as the first, except that the beam intensity was cut by a factor of four and the exposure was twice as long, resulting in the reduction of the total dose by half. The results of this run were similar to those of the first, except that the lower molecular weight molecules were enriched relative to the higher molecular weight ones. This indicates an 'evolutionary' sequence from low molecular weight to higher molecular weight, but further work will be done to confirm this.

A 1 : 1 : 1 mixture of $CH_4 : NH_3 : H_2S$ at a total pressure of 605 mm Hg was used in the third experiment. Before the irradiation, a thin white haze (NH_4SH ?) was observed inside the cells, but it was not seen after the exposure. This seems to be analogous to the white residue noticed by Sagan and Khare[8] in one of their experiments. The gas phase contained several simple hydrocarbons, saturated and unsaturated, along with CH_3CN and $(CH_3)_2S$. The gas chromatogram of the liquid, which was much yellower than in the previous cases, indicated the presence of a series of alkyl di and tri-sulphides, starting with $(CH_3)_2S_3$ and increasing in alkyl chain length, as deduced from the mass spectra of the various fractions. The yellow colour was found to be free polymeric sulphur, predominantly S_8. This is a very interesting result, as free sulphur (and subsequent ammonium polysulphides) have been proposed to account for the yellow and yellow-brown coloured layers on Jupiter[14]. Noticeably absent were the cyclic amines produced in the $CH_4 : NH_3$ experiments; the exposure here was a relatively short one and the result may reflect the relative reactivities of H_2S and NH_3.

In experiment four, a mixture of CH_4 with nitrogen was used instead of CH_4 and NH_3. The gas phase contained the same species as in the NH_3 cases but the liquid, similar in appearance to the others, contained a much larger proportion of alkyl amines relative to the cyclic amines.

The last cell, containing only CH_4, was run as a check on possible contributions of the apparatus itself to the final irradiation products. The gas phase again contained simple hydrocarbons, but also a trace of NH_3, caused by the nitrogen remaining in the cell after pumping and filling. The clear non-viscous liquid formed contained a mixture of higher molecular weight hydrocarbons, both saturated and unsaturated. No traces of any compounds containing nitrogen or sulphur were found.

It is obvious from the above results that energetic protons can be an effective energy source for the formation of complex molecules from simple ones. Subsequent decomposition of the products is not as serious a problem as might be believed as there are likely to be processes, such as convection and condensation, in the atmospheres for removing the molecules from the reactive zone, and there were found to be fair amounts of the liquids formed on the rear windows of the cells in the centres of the proton beams. In spite of the extreme energies involved (2 MeV) as compared to typical bond energies, there seems to be some sensitivity to different initial reactants, as different results were obtained for the $CH_4 : NH_3$ and CH_4N_2 mixtures, both containing only C, N and H. With the exception of the sulphide compounds none of the molecules that were identified are coloured; however, the colouring agents could be contained in the unresolvable fractions. It should be noted that no coloured compounds were produced in the pure CH_4 experiment; the presence of N or S seems to be necessary for their formation. Future experiments are being designed to probe this and other aspects relative to the outer Solar System colouring problem.

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Particle Formation from NH_3-SO_2-H_2O-Air Gas Phase Reactions

RECENT articles on the formation of atmospheric aerosols from NH_3–SO_2–H_2O gas phase reactions[1,2] have considered only those products formed between the anhydrous gases to give 2:1 and 1:1 (NH_3:SO_2) compounds. I wish to draw attention to my work[3] on the reaction between these gases at partial vapour pressures in air of 50 to 500 N m^{-2}. Solid products were formed only in the presence of water vapour at 20° C. Analyses showed that the compounds formed were either ammonium sulphite (with excess NH_3) or ammonium pyrosulphite (with excess SO_2). Vapour pressure measurements at 0 to 23° C indicated that the extrapolated vapour pressures of NH_3 and SO_2 above the sulphite in dry air at −70° C would be about 1×10^{-4} and 5×10^{-5} N m^{-2} respectively. With 50% saturation of the air with water vapour at −70° C, however, these vapour pressures would be depressed to 8×10^{-6} and 4×10^{-6} N m^{-2} respectively, that is, to concentrations approximately equal to those reported for the 2:1 compound. The partial vapour pressures of the pyrosulphite under similar conditions would be 3.3×10^{-5} N m^{-2} for both gases. At −52° C the vapour pressures of these gases in dry air would be identical for both the 2:1 compound and ammonium sulphite.

It seems, therefore, that there will be competition between the formation of the 2:1 compound and ammonium sulphite in the stratosphere except under completely anhydrous conditions.

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Thorium Determination in Glasses using Fission Track Technique

WE have applied the fission track technique to the determination of thorium in glasses. Neutron-induced fission occurs for thorium and uranium, the isotopes ^{232}Th, ^{235}U and ^{238}U fissioning with fast neutrons, and ^{235}U with thermal neutrons. Two irradiations are performed. The first irradiation is with predominantly thermal neutrons for determination of the uranium content (comparing to a standard and knowing the isotopic ratio of uranium) and the second irradiation is with predominantly fast neutrons which gives the uranium and thorium contents combined. The 'track excess' from the second irradiation, when the unknown is again compared to a uranium standard, is attributed to thorium fission.

Consideration of several factors is important for evaluation of a standard. Knowledge of (1) the fission cross sections for the various isotopes as a function of neutron energy[1], (2) the neutron energy spectrum for the reactor facility[2] and (3) the isotopic concentrations enables the percentage of tracks contributed by any one isotope to be calculated. The total number of fissions produced ($\Sigma_\tau \Phi_\tau$) from a mixed neutron energy spectrum, where the fast neutron energy spectrum is taken between 1 and 10 MeV and the thermal neutron induced fission of only ^{235}U is considered, is given by

$$\Sigma_\tau \Phi_\tau = N^{232}\int_1^{10}\sigma_f^{232}(E)\,\phi_f(E)\,dE + N^{238}\int_1^{10}\sigma_f^{238}(E)\,\phi_f(E)\,dE + N^{235}\int_1^{10}\sigma_f^{235}(E)\phi_f(E)dE + N^{235}\sigma_t^{235}\phi_t$$

where N is the atom density of an isotope; $\sigma_f(E)$, the fast fission cross section of a specific isotope as a function of energy; $\phi_f(E)$, fast neutron flux as a function of energy; dE, energy interval; σ_t, the cross section for thermal fission of ^{235}U; and ϕ_t, the thermal neutron flux.

Table 1 Comparison of Fission Tracks and Other Techniques for Determination of Thorium

Sample	Th (p.p.m. by weight) *	U (p.p.m. by weight)	U isotopic ratio 8/5	Th (other method, p.p.m. by weight)	Density (g cm^{-3})
La 1	87±9	0.015±0.002 †	Natural	76 ‡	3.60
La 2	265±33	— §	Natural	—	3.56
La 3	466±56	0.015±0.002 † ‡	Natural	452 ‡	3.58
La 4	712±60	— §	Natural	—	3.61
La 5	920±65	— §	Natural	—	3.55
T-1	31.5±1.0	0.046 †	189	31 ‡ 40.6±0.8 * *	2.28
K2a	0.42±0.04	0.345±0.083 ‖ ¶	Natural	0.352±0.008 ¶	2.03
614 SRM		0.823 † ‡	387	0.746 ‡	2.55

* Errors are one standard deviation based on track counting statistics.
† Nuclear track determination.
‡ Isotopic dilution mass spectrometry (all measurements by W. R. Shields *et al.* ±0.25% standard deviation).
§ Since all the lanthanum glasses were made from the same base melt, it was assumed that the U content did not vary for the series of glasses.
‖ X-ray fluorescence.
¶ Neutron activation analysis.
* * Using α irradiation, see ref. 9.

Therefore, from this equation and with our irradiation conditions with predominantly fast neutrons[3], and for a material containing the natural isotopic ratio of uranium (^{238}U/^{235}U = 137.8), a Th/U ratio of 1,000 would mean that 80% of the tracks are from Th fission; a Th/U ratio of 4 means that only about 3% of the tracks are from Th fission. This low percentage approaches the limit of error imposed by track counting statistics. In our experiments, the standard used was NBS SRM 614 (ref. 4) where Th/U = 0.906 and ^{235}U = 0.2792 atomic % abundant. Even with the depleted ^{235}U, this served well as a uranium standard in the fast neutron facility and only 1.5% of the tracks observed in the detector were from thorium fission.

The samples and standard were mounted in epoxy and polished; an external detector was then affixed. Mica was preferred for use as an external detector because the temperatures attained during the second irradiation with fast neutrons would partially anneal the tracks in a plastic detector.

The glasses used in this study consisted of five lanthanum-based glasses (~48% La$_2$O$_3$) used as thorium standards by the US Army Night Vision Laboratory at Fort Belvoir, Virginia. Thorium glass T-1 described by Schreurs and others[5], and glass K2a described by Lovering (personal communication). The fission track results compared to Th determination by other methods (where data are available) are shown in Table 1. It was assumed that the range of fission fragments is proportional to the density of the material through which they pass[6]. Therefore, an additional correction was made for the differences in densities of the standard glass and the unknown glasses. As expected, the error increases with a lower Th/U ratio. For glass K2a with a Th/U ratio of ~1, our result was 20% greater than the value reported by Lovering. The fission of ^{235}U is still a major contributing factor, however, and by reducing this contribution by cadmium shielding, for example, or in a facility with a greater fast flux component, the error would be reduced.

This particular technique can easily be adapted for Th determination in minerals where the natural U isotopic ratio can be assumed, and would permit data to be acquired that would be more accurate than previously obtained by a neutron induced fission track method[7]. In addition, using this fission track technique for determining thorium and uranium avoids the uncertainties which result from beam current heating of the sample or observation efficiency differences when either α or other high energy particles are used to induce fission of thorium[8,9].

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NaOH Etch of Fission Tracks in a Soda-Lime-Silica Glass

IT is generally known that fission tracks intersecting the surface of man-made soda-lime-silica glasses can be etched with HF at room temperature[1,2]. In this technique the acid attacks the tracks preferentially so that they become visible in the microscope before the glass itself is destroyed. An NaOH etch at 60° C has been reported for phosphate glasses[3].

I wish to report a glass which I believe to be a soda-lime-silica glass that is etched by NaOH rather than HF. The material consists of numerous glass beads of a recognisable type known as 'trade wind beads' found in archaeological sites of the past millennium in eastern and southern Africa. They are consistently similar in appearance and chemical composition. Their composition is partly reported in Table 1 and abundant further information is available elsewhere[4].

Table 1 Partial Composition of 'Trade Wind Beads'

Element	Concentration	Method	No. of samples
Na	15.0±1.5%	NAA	133
Ca	4.4±1.9%	NAA	133
K	2.3±1.4%	NAA	133
Pb	~0.01–7.0%	XRF	~200
P	~0.1%	ES	2
U	112±55 p.p.m.	NAA	133
(SiO_2	~56%	XRF	1)

NAA, neutron activation analysis; XRF, X-ray fluorescence analysis; ES, emission spectroscopic analysis.

The beads are thought to contain fossil fission tracks useful for dating. In an attempt to etch such tracks, experiments were carried out on artificially produced fission tracks in 'trade wind beads'. HF at various concentrations at room temperature attacked the glass without etching the tracks preferentially. Successful etching was accomplished by refluxing for 7 min in NaOH. The reflux solution was made by dissolving 12 g NaOH in 16 g water.

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BIOLOGICAL SCIENCES

Proximal Myopathy induced by 5-HT-Imipramine simulates Duchenne Dystrophy

IT has recently been observed that patients with Duchenne muscular dystrophy (DMD) have a significant defect in the uptake of serotonin (5-HT) into platelets[1], raising the possibility that a defect in the metabolism of biogenic amines may be important in the pathogenesis of the skeletal muscle lesions of this disorder. If monoamines such as 5-HT or norepinephrine (NE) which are dependent on the intracellular enzyme, monoamine oxidase (MAO), have a diminished ability to reach this site of metabolism, increased tissue levels of these compounds could result and produce skeletal muscle damage. The effect of such a defect would be enhanced in skeletal muscle as catechol-O-methyltransferase, the alternative pathway for metabolism of biogenic amines, is absent from this tissue[2]. Support for this hypothesis of skeletal muscle damage is found in the observation that skeletal muscle from biopsies of Duchenne dystrophy patients has shown the presence of an abnormal intrafibrillar fluorescence (Falck-Hillarp technique) presumed to be a monoamine-like substance[3]. Furthermore, injection of the MAO inhibitor, pargyline, results in a myopathy with evidence for accumulation of monoamines[4]. The pargyline induced myopathy, however, has serious limitations as a model for Duchenne dystrophy, as the lesions are limited to the soleus muscle and, furthermore, MAO activity has been found to be normal in patients with DMD (ref. 1).

Here we report the findings of an experimental myopathy using 5-HT in combination with imipramine, a pharmacological agent blocking uptake of biogenic amines into platelets[5] and thereby producing a defect in biogenic amine metabolism closely simulating that observed in patients with DMD. The results of this study are extremely important in two ways. First, the very typical early and mid-stage histological lesions of DMD were reproduced and, second, this study represents the first demonstration of an experimental animal model with skeletal muscle lesions predominating in the proximal musculature, as is so characteristic of the clinical disease.

Fifty male Osborne-Mendel and Sprague-Dawley rats weighing 135 to 150 g were used. Fresh frozen sections of soleus, gastrocnemius, anterior tibialis, extensor digitorum longus, quadriceps, iliopsoas and deltoid muscles were stained by modified trichrome, haematoxylin and eosin, reduced nicotinamide dinucleotide-tetrazolium reductase and myofibrillar adenosine triphosphatase[6]. At the beginning of the study all rats were injected intraperitoneally with imipramine, 10 mg kg^{-1}, for 3 d. On the fourth day the animals received 5-HT, i.p., 100 mg kg^{-1}. Each week two rats were killed 4 d after the injection of 5-HT, the remaining rats being injected with the same dosage schedule for a total of 11 weeks.

Skeletal muscle lesions were seen in all rats examined and increased in severity as the number of injections in each rat increased. The distribution of the histological lesions in these animals was quite striking as the lesions were very severe in the quadriceps, moderately severe in iliopsoas and occasionally present in the soleus. In the gastrocnemius, anterior tibialis and extensor digitorum longus, only rare skeletal muscle pathology was encountered. The earliest lesions consisted of necrosis and phagocytosis and regeneration of individual muscle fibres or small groups of muscle fibres surrounded by a field of normal muscle (Figs 1 and 2). This is identical to the early lesions of DMD (ref. 7). In animals receiving multiple injections, the lesions showed signs of chronicity manifested by a marked variability in fibre size, internal sarcolemmal nuclei and proliferation of connective tissue surrounding individual muscle fibres (Fig. 3). This is characteristic of the mid-stage histological lesions of DMD (ref. 7). No abnormalities were seen in the intramuscular nerves and the intramuscular arteries showed no signs of occlusion, although, rarely, they were surrounded by a small round cell infiltrate, similar to that described in DMD (ref. 8).

Control animals included those injected i.p. with imipramine 10 mg kg^{-1} as above but without follow-up injections of 5-HT. Another group of animals was injected i.p. daily for 7 d with imipramine, 25 and 50 mg kg^{-1}. A third group of controls included those injected with single doses of 5-HT, 100 mg kg^{-1}. Only rare scattered fibres undergoing necrosis and phagocytosis were seen in the animals injected with imipramine, 50 mg kg^{-1} and 5-HT, 100 mg kg^{-1}.

We feel that this animal model of Duchenne dystrophy is extremely important because not only have the highly characteristic histological skeletal muscle lesions been reproduced but of possibly greater significance is the fact that the proximal muscles were found to be the principal site of lesion predilection. These abnormalities were the result of injections of 5-HT in combination with a pharmacological agent, imipramine, which reproduces an abnormality in biogenic amine metabolism similar to that found in patients with DMD. This study adds significance to the previously described transport defect of 5-HT (ref. 1) in DMD and opens many new avenues of investigation in a disease which has remained an enigma since its description. Furthermore, this 5-HT-imipramine induced myopathy provides a suitable animal model for future study which is superior to others previously described using arterial embolisation[8] or aortic ligation in combination with 5-HT or NE (ref. 7). These models, requiring surgical manipulation, failed to show a predilection of skeletal muscle pathology in a proximal distribution.

The pathogenesis of the proximal muscle lesions produced in this animal model remains to be elucidated. It is unclear at this time whether the lesions are the result of functional ischaemia to the skeletal muscle or are the result of direct myotoxicity. It also remains to be established whether or not these lesions are dependent on an intact nerve supply as are those induced by the MAO inhibitor, pargyline. Studies are currently under way to answer these and other questions.

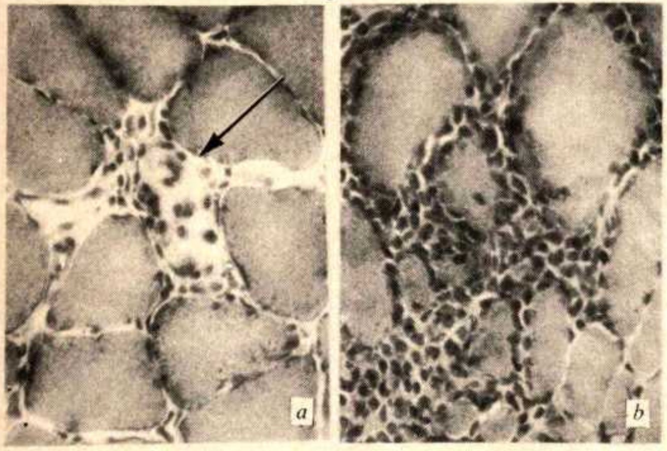

Fig. 1 *a*, Single muscle fibre undergoing necrosis and phagocystosis (arrow) in soleus. (×150.) *b*, Small group of muscle fibres undergoing necrosis and phagocytosis in quadriceps. (×280.)

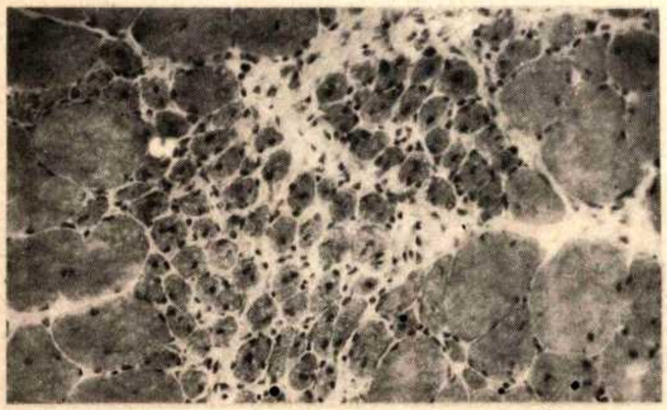

Fig. 2 A group of small regenerating muscle fibres in quadriceps surrounded by normal fibres. Note the enlarged central nuclei characteristic of regeneration. (×155.)

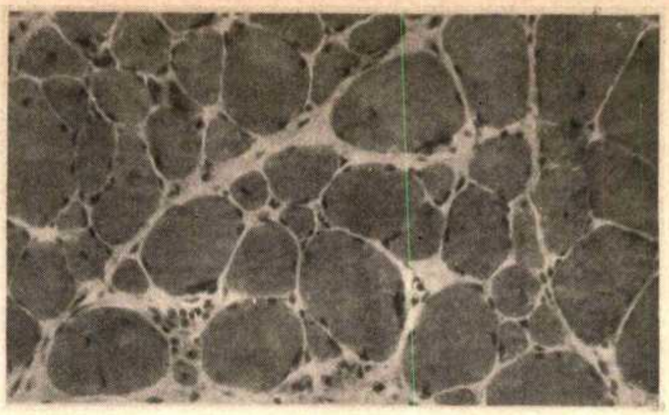

Fig. 3 A group of muscle fibres of variable size, many with internal nuclei, and proliferation of endomysial connective tissue between individual fibres. (×194.)

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Dose Dependence of Antigen Binding to B and T Lymphocytes

Specific precursor cells, as lymphocytes binding ^{125}I-labelled antigens, can be detected by autoradiography (see refs 1 to 3 for reviews). Classification of such antigen binding cells (ABC) into T and B cells gave equivocal results. Cell populations enriched in B cells (by treatment with anti-θ serum and complement) or in T cells (by passage through anti-immunoglobulin columns) indicated that some of the ABC were indeed T cells, but that they bound far less antigen than did B cells[4–7]. Using a direct approach by treating lymphocytes in sequence with ^{125}I-antigen (*Maia squinado* haemocyanin: MSH, or bovine serum albumin) followed by fluorochrome-labelled anti-immunoglobulin, anti-B, anti-T, or anti-θ reagents, Lamelin *et al.*[8] did not detect significant numbers of T ABC, while Roelants *et al.*[9] using the same antigen MSH and also the heavily iodinated (>50 atoms I per molecule) (Tyr, Glu)-Ala-Lys synthetic polypeptide (TIGAL) found that 20–30% of the ABC in primed mice were T lymphocytes and that they bound as much antigen as B ABC. Joint experiments (J. P. Lamelin and G. E. Roelants, unpublished) showed that this discrepancy was due to the hundred-fold lower concentration of antigen used by Lamelin *et al.* The influence of antigenic concentration on the binding by B and T cells has been systematically investigated, with the following results.

Antigen binding, with detection of immunoglobulin or θ surface markers on the same cell, has been described[9]. Briefly, 20×10^6 spleen or lymph node cells were mixed with various amounts of ^{125}I-TIGAL (20 to 300 μCi μg^{-1}) in a final volume of 0.4 ml ice cold Eagle's Minimal Essential Medium, 10% foetal calf serum (FCS), 1.5 mM NaN$_3$. In preliminary experiments cells were also exposed to antigen (2.5 μg ml^{-1}) at higher temperatures (23° C and 37° C) which only had a minimal effect on ABC frequency (10–20% increase of both B and T ABC). Further experiments were always carried out in the cold. After 45 min the cells were washed of excess antigen, incubated with either rhodamine-conjugated rabbit anti-mouse Ig or AKR anti-θ C3H immunoglobulin, washed, smeared on gelatine-coated slides and fixed for 5 min in absolute ethanol. Autoradiography was performed using Ilford K5 emulsion. The cells were observed under phase contrast at 100×6.3-fold magnification with Leitz Orthoplan microscopes equipped with Osram HBO-200 mercury vapour lamps and Opak-Fluor vertical illuminators (E. Leitz GmbH, Wetzlar, Germany). B lymphocytes were identified as immunoglobulin positive or θ negative and T lymphocytes as immunoglobulin negative or θ positive. The validity of this classification has been discussed in detail[9].

Lymph nodes or spleen cells from unprimed C3H/He mice (either sex, 2.5 to 5-month-old, Roche, Füllinsdorf, Switzerland) or spleen cells from primed mice (100 μg TIGAL precipitated with alum and 10^9 killed *Bordetella pertussis* intraperitoneally 4 month previously) showed a plateau in the number of B and T ABC at antigen concentrations of about 2 μg ml^{-1}. Cells pooled from 4–6 mice were used for each experiment. In accordance with reported results[9] at the plateau the overall frequency of ABC 4 month after priming was not very different from that of unprimed mice (400×10^{-5} to 700×10^{-5}). Figure 1 shows, for example, the ABC frequency of normal lymph node cells exposed to concentrations of TIGAL ranging from 0.5 to 4 μg ml^{-1}. Earlier experiments over the range of 2 to 64 μg ml^{-1}, at two-fold steps, did not cover the lower limit of the curve but showed that the plateau extended over all concentrations above 2 μg ml^{-1}, so the rise at 4 μg in Fig. 1 was not significant.

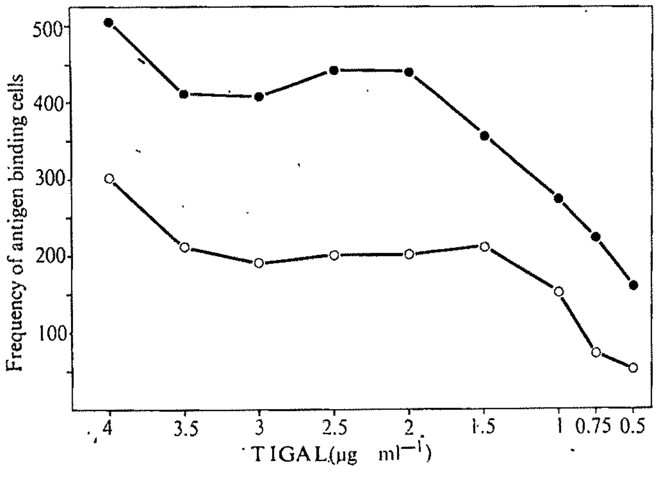

Fig. 1 Frequency of antigen binding B (●, $\times 10^{-5}$) and T (○, $\times 10^{-6}$) cells in a normal lymph node cell population at various concentrations of antigen (50,000 to 100,000 lymphocytes were examined per group).

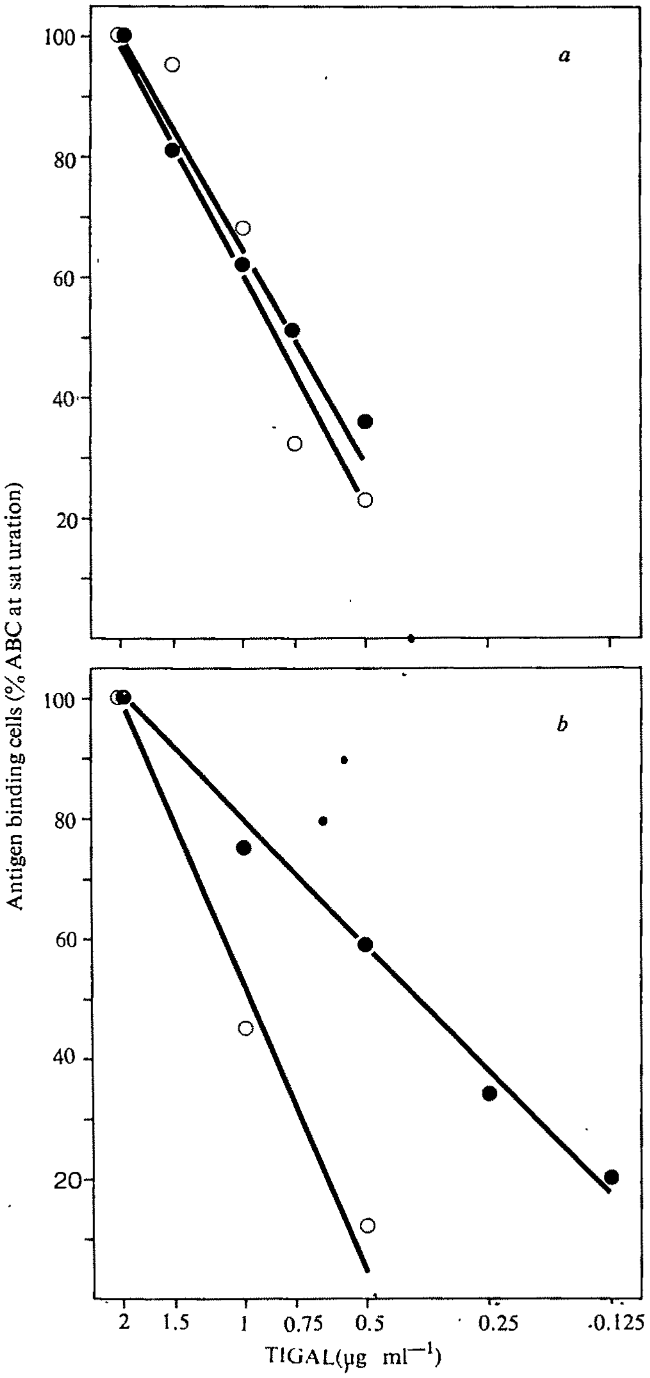

Fig. 2 Titration of, *a*, normal and, *b*, TIGAL-primed antigen binding cells at antigen doses below the plateau level (50,000 to 100,000 lymphocytes were examined per group). ●—●, B; ○—○, T lymphocytes.

Figure 2 shows the regression of B and T ABC on antigen concentrations below the saturation level. For unprimed cells the slopes are similar for B and T ABC; the slope of B ABC decreases after priming. This observation, in agreement with those of Möller and co-workers[10,11] using hapten inhibition of rosette formation, indicates that the avidity of both T and B rosette-forming cells is similar in unprimed animals, whereas after priming the avidity of B cells is increased, but that of T cells remains unchanged.

The degree of labelling, as indicated by the number of grains, was similar for T and B cells at a TIGAL concentration of about 2 μg ml^{-1}. Varying the concentration of antigen had a strikingly different effect on the degree of labelling of B or T ABC. As exemplified in Fig. 3 with a primed spleen cell population, the proportion of heavily

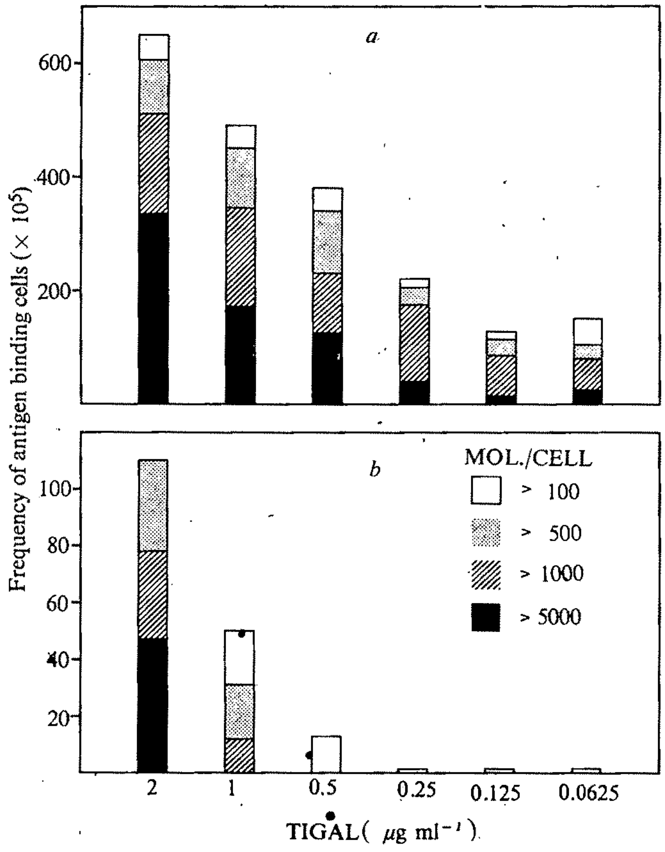

Fig. 3 Degree of labelling of TIGAL-primed antigen binding cells at various antigen doses. The ABC were distributed into four classes depending on their degree of labelling expressed as molecules per cell (see inset legend). *a*, B cells; *b*, T cells.

labelled B cells decreased when the concentrations of antigen were lowered, but even at the lowest concentrations used (0.0625 μg ml^{-1}) some heavily labelled B cells were still present. In sharp contrast all the heavily labelled T cells were lost by a mere two-fold dilution from the plateau (1 μg ml^{-1}). Unprimed cells behaved the same way, even though the overall proportion of T and B cells labelled was similar at different concentrations of antigen (see Fig. 2). Since the number of B ABC did not increase between 4 and 64 μg TIGAL ml^{-1}, some B cells were saturated with as little as 100 molecules of antigen. T cells seemed to be more homogeneous in this respect since they all become heavily labelled at high concentrations of TIGAL. Whether all T ABC bind a maximal amount of antigen (about 50,000 molecules) at saturation cannot be decided from these experiments, due to overlapping and confluent grains above 500 per cell.

These observations render it mandatory to establish binding curves over a wide range of concentrations. Without this tedious process, no valid information about the number of B and especially T ABC can be obtained. Hämmerling *et al.*[6,7] studying ABC for (T,G)-A--L (antigenically different from TIGAL) used concentrations of antigen between 0.5 and 1 μg ml^{-1} and detected only T ABC binding less than 500 molecules of antigen. Even with the far more potent immunogen TIGAL used here no heavily labelled T ABC are detected at those antigen concentrations. Binding curves for (T,G)-A--L are being determined and preliminary results indicate that a plateau is reached only for antigen concentrations as high as 25 μg ml^{-1}.

As shown by the saturation experiments, T cells possess a number of receptors for antigen comparable to that of B cells. The effect of antigen dilution on the distribution of label density is, however, far more pronounced for T than for B ABC, indicating that the average avidity for antigen is both lower and more uniform. Whether this is due to an inherent property of the receptor or is due to the nature of the T cell membrane remains to be investigated. The fact that even primed B ABC have widely varying numbers of receptors suggests that the high avidity of primed B ABC may be due not only to selection of cells with higher affinity receptors but also to 'maturation' of cells acquiring a larger number of receptors. Neither selection nor maturation could be discovered for T ABC.

The titration curves of this study do not argue either for or against the immunoglobulin nature of the T-cell receptor. Experiments approaching this problem in a direct way are presented in a companion paper[12].

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Active Synthesis of Immunoglobulin Receptors for Antigen by T Lymphocytes

WHEN antigen binding cells (ABC) were studied by combined autoradiography and immunofluorescence it was found that antigen binding to both B and T ABC could be inhibited by anti-immunoglobulin (anti-Ig) reagents[1]. Moreover, receptors for antigen could be redistributed in caps in a similar way for B and T ABC using non-inhibiting doses of anti-Ig reagents[1]. Since membrane components are known to redistribute independently of each other[2–5] it was concluded from the inhibition and capping experiments that the receptor for antigen on T lymphocytes was immunoglobulin. The fact that T ABC are always a small minority of the total T cell population argues against the possibility that their receptors are passively acquired as cytophilic antibody. We present evidence confirming that T cells have receptors for antigen which are immunoglobulins and showing, in addition, that they are actively synthesised by the cells carrying them.

Both the redistribution of surface immunoglobulin after interaction with anti-Ig reagents, and the combined autoradiographic immunofluorescent technique of estimating antigen binding and demonstrating immunoglobulin or θ surface markers on the same cell, have been described previously[1,3]. Our present experimental scheme is set out in Fig. 1. Lymph node cells (80 to 85% T cells) from four to six unprimed C3H/He mice per experiment (either sex, 2.5 to 5 months old, Roche, Füllinsdorf, Switzerland) were exposed to a polyvalent rabbit-anti-mouse immunoglobulin reagent labelled with fluorescein isothiocyanate (FITC-RIgG-α-MIgG) and incubated under conditions allowing polar redistribution (capping) and endocytosis of cytoplasmic membrane immunoglobulin. In some experiments a second layer of unlabelled sheep anti-rabbit L chain reagent (Sser-α-RL)[3] was added to ensure complete clearing of surface Ig. After various incubation times, samples of cells were taken and exposed, under non-capping conditions, to radioactively labelled heavily iodinated (Tyr, Glu)-Ala--Lys synthetic polypeptide (> 50 atoms I per molecule) (^{125}I-TIGAL) at saturating doses for detection of ABC[6]. The cells were then divided into two parts under non-capping conditions: one was stained with rabbit anti-mouse immunoglobulin (IgG) reagent labelled with tetramethylrhodamine isothiocyanate (TRITC-RIgG-α-MIgG)[3], the other with rhodamine-labelled AKR anti-θ-C3H IgG (TRITC-anti-θ)[1,7]. As a control, cells were first incubated with a rabbit IgG antibody directed against tobacco mosaic virus (FITC-RIgG-α-TMV)[3] instead of FITC-RIgG-α-MIgG; subsequently these cells went through the same steps as the experimental group.

(1) Normal lymph node cells in RPMI 1640 medium 0.03 M HEPES; 10% foetal calf serum (FCS), 2 mM glutamin, penicillin-streptomycin, pH 7.2

+FITC-RIgG-α-MIgG 250 μg ml^{-1} +FITC-RIgG-α-TMV (control) 250 μg ml^{-1}

0° C, 10 min } 50 × 10^6 cells ml^{-1},
37° C, 50 min } medium without NaN$_3$

wash 3 ×

(2) (Optional) +Sser-α-RL
1/40 final dilution
37° C, 15 min, 50 × 10^6 cells ml^{-1},
medium without NaN$_3$

wash 3 ×

(3) Incubate at 37° C, for variable lengths of time, 0.5 × 10^6 cells ml^{-1}, medium without NaN$_3$, 4 ml in Falcon Tissue Culture Flasks 3012.

wash 2 × in medium with NaN$_3$ (1.5 mM), 0° C.

(4) +^{125}I-TIGAL (200–468 μCi μg^{-1}) 2.5 μg 25 × 10^6 cells ml^{-1}

0° C, 30 min, medium with NaN$_3$ (1.5 mM)

wash through FCS gradients

(5) +TRITC-RIgG-α-MIgG 250 μg ml^{-1} +TRITC-anti-θ 250 μg ml^{-1}

0° C 30 min,
50 × 10^6 cells ml^{-1},
medium with NaN$_3$ (1.5 mM)

(6) Wash 3 ×, smear on gelatine coated slides, fix in absolute ethanol (5 min), process for autoradiography (Ilford K5 emulsion).

Fig. 1 Basic experimental procedure.

Characterisation of the various reagents used has been described[1,3,7]. The main specificity controls were as follows. TRITC–anti-θ stains the expected proportion of C3H mouse lymph node, spleen or thymus lymphocytes but stains neither AKR mouse thymocytes nor nude mouse spleen cells. Its capacity to stain C3H thymocytes or spleen cells is completely removed by absorption with C3H mouse brain. The capacity of TRITC-RIgG-α-MIgG to stain B cells, to block or to induce polar redistribution of receptors for antigen on T and B cells is removed by absorption with unsolubilised IgG myeloma. We used the FITC conjugate of the same RIgG-α-MIgG preparation. The capacity of both TRITC and FITC-RIgG-α-MIgG to stain B lymphocytes is absorbed by passage on electrophoretically purified IgG from normal mouse serum coupled to Sepharose. Anti-lymphocyte serum does not inhibit antigen binding to B and T lymphocytes and does not redistribute in polar caps, indicating that the activity of our reagents is not simply due to anti-membrane contamination.

The basic experiment was repeated five times, always using normal lymph node cells to exclude cytophilic anti-TIGAL antibody. The findings were reproducible and a sample is given in Fig. 2 (see also Table 1).

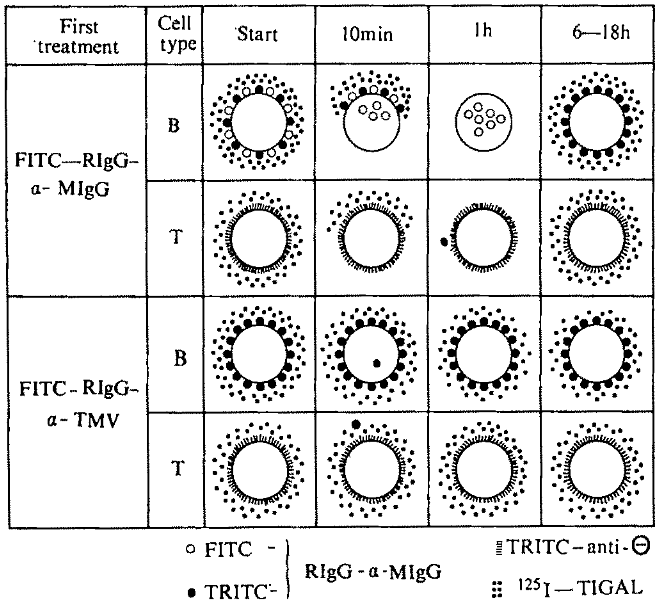

Fig. 2 Capping and resynthesis of immunoglobulin receptors for antigen on T and B lymphocytes. For experimental protocol see Fig. 1 and text.

FITC-RIgG-α-MIgG induced capping and endocytosis of cell surface Ig as detected on B cells by fluorescence microscopy (Leitz Orthoplan microscopes equipped with Osram HBO-200 mercury vapour lamps and Opak-Fluor vertical illuminators; 100 × 6.3 or 100 × 1.25 × 6.3 magnifications[1,3]). Endocytosis was complete in about 1 h. At this point the cells were no longer stained by the subsequent treatment with TRITC-RIg-Gα-MIgG. As described before[1], during the capping treatment both B and T ABC at first showed ^{125}I-TIGAL radioactive grains in caps, superimposed on the Ig caps in the case of B ABC, but after about 1 h, neither B nor T ABC were detected. In three experiments, ABC and surface immunoglobulin started to reappear about 3 h after capping and the receptor resynthesis was complete in about 6 h, in agreement with earlier observations[3]. In two experiments, however, complete resynthesis took 12–18 h. It is noteworthy that T ABC in each case followed the same kinetics of disappearance and reappearance as B ABC.

It would seem far-fetched that, in a population of normal lymph node cells, rare T cells (frequency 23–43 × 10^{-5}) would bear at their surface enough passively adsorbed antibody to bind up to 50,000 molecules of TIGAL and even less likely that during the *in vitro* incubation after capping, TIGAL-specific B cells (frequency 54 × 10^{-5} to 108 × 10^{-5}) would have secreted enough anti-TIGAL antibody which would bind only to those rare T ABC to bring their number back to their initial level. It could still be argued that the antigen-specific T-cell receptors were associated with some passively acquired Ig, were co-capped with it by the anti-Ig treatment, and were

Table 1 Active Synthesis of Ig Receptors for Antigen by B and T Lymphocytes

	Proportion of B cells in total population	ABC frequency $\times 10^{-5}$ *		Groups compared	Statistical analysis *P* value †	
		B	T		B	T
1 Untreated population	17%	69	28	1 : 2	<0.0005	<0.01
2 1 h after first capping with RIgG-α-MIgG	17%	0	0 ‡	2 : 3	<0.001	<0.005
3 18 h after first capping with RIgG-α-MIgG	9%	25	21	3 : 4	<0.001	<0.005
4 1 h after second capping with RIgG-α-MIgG	7%	0	0	1 : 3	<0.025 >0.40§	>0.60
5 1 h after RIgG-α-TVM	17%	84	47	1 : 5	>0.50	>0.30
6 18 h after RIgG-α-TVM	10%	23	21	1 : 6	<0.025 >0.50§	>0.50

* 50,000 to 75,000 lymphocytes were examined per group.
† Based on χ^2 test.
‡ One cell was found with ~thirty grains still in a small cap.
§ When corrections were made for the decrease in the overall proportion of B cells, which was found in two experiments out of five.

later resynthesised. Although there is no precedent for such co-capping, we sought formal proof that the antigen binding receptors which reappeared on B and T ABC were indeed immunoglobulin.

Two experiments were therefore extended by taking samples of cells known to have capped and resynthesised and exposing these cells once again to FITC-RIgG-α-MIgG. They were then, as before, put in capping conditions and later exposed, under non-capping conditions, to ^{125}I-TIGAL, followed by TRITC-RIgG-α-MIgG. Results of one complete experiment are given in Table 1: receptors for antigen on both B and T ABC which had been resynthesised after capping were cleared again by a second treatment with anti-Ig reagents. Samples of cells were examined before the total clearance of surface receptors by the second FITC-RIgG-α-MIgG treatment and showed that this clearance was also due to capping.

These experiments demonstrate the active synthesis of immunoglobulin receptors for antigen by T lymphocytes. The nature of these receptors in terms of known Ig classes remains to be investigated. It is perplexing that these receptors are not readily visualised using conventional fluorescent anti-Ig reagents. This may be due to their location in the T cell membrane; for instance, only a small part of the Ig receptor molecule(s) may be accessible to rabbit anti-M IgG antibody. These parts may, however, be detectable by antibody present in anti-M Ig antisera raised in chicken (F. L., J. R. L. Pink and L. Du Pasquier, manuscript in preparation).

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Increase in Kallikrein Excretion during the Natriuresis produced by Arterial Infusion of Substance P

SUBSTANCE P was discovered by von Euler and Gaddum in 1931 in extracts of brain and small intestine[1] and its distribution and effects have recently been reviewed[2]. Its structure has been identified[3] and the undecapeptide has recently been synthesised[4]. Substance P is a potent stimulator of salivary secretion[5]; stimulation of the chorda-lingual nerve leads to increases in the production of saliva and blood flow through the salivary gland and is associated with the depletion of glandular kallikrein[6]. The presence of atropine blocks secretion but does not affect the increase in blood flow[7] or kallikrein depletion[6]. In homogenised nervous tissue substance P is located in the fraction containing the synaptic vesicles[8].

It seemed from these findings that substance P at the nerve endings might be released and lead to the activation or release of kallikrein. Kallikrein has been demonstrated in a variety of tissues which produce a liquid secretion such as salivary glands[9], small intestine[10], pancreas[11], sweat glands[12] and kidneys[13]. Urinary kallikrein resembles renal kallikrein[14] and differs from plasma kallikrein[15]. In severe renal failure urinary kallikrein excretion is very high which also supports the view that it is of renal origin and not filtered from plasma[16]. Kallikrein excretion is closely related to sodium excretion in a variety of circumstances[16–18]. Nevertheless increased sodium excretion also occurs without an associated increase in kallikrein excretion when there is a sharp fall in mineralocorticoid activity. These two processes related to sodium excretion are clearly differentiated by the Na/K ratio in the urine[19].

If 'substance P-inergic' nerves exist and promote the release of kallikrein, it might be expected that substance P infusion into the renal artery would lead to the release of kallikrein and a natriuresis. This has been tested in greyhound dogs under pentobarbital anaesthesia. Both ureters were catheterised and 0.9% saline was infused into the left renal artery at 1 ml min^{-1}. After several 10 min control collections of urine the infusion was changed to synthetic substance P (Beckman) in saline for three periods, then back to saline alone. Urinary sodium was measured by flame photometry. Kallikrein was assayed by determining the esterase activity of the enzyme in releasing tritiated methanol from a synthetic labelled substrate (*p*-tosyl-L-arginine-^{3}H-methyl ester)[20]. Pancreatic kallikrein (Bayer) was used as a standard in the assay and the results are given in esterase units, which are defined in Table 1.

In thirteen experiments on five dogs doses of 1, 10 and 100 ng substance P min^{-1} were used, equivalent to 30–4,300 pg kg^{-1} min^{-1}. The changes in sodium and kallikrein excretion, calculated as the differences between excretion in the experimental periods and the mean of the control periods immediately preceding and following the peptide infusion, are shown in the

Table 1 Increases in Sodium and Kallikrein Excretion in Response to Arterial Infusions of Substance P

Substance P dose (ng min^{-1})	Sodium excretion (μEq min^{-1})		*P*	Kallikrein excretion (mEU min^{-1})		*P*
	Control	Increase		Control	Increase	
1	38.8± 9.67 n (8)	11.7± 4.35 (12)	<0.025	411± 87 (8)	12±27 (12)	NS
10	43.8± 9.64 n (10)	67.3±14.73 (15)	<0.001	395± 98 (10)	127±33 (15)	<0.005
100	55.0±14.27 n (8)	128.9±19.16 (12)	<0.001	443±123 (8)	192±46 (12)	<0.005

Means ±s.e.
n, Number of control or experimental periods (see text).
P is derived from a *t* test on paired values.
EU, Esterase unit, equivalent to the hydrolysis of 4.8 μmol *p*-tosyl-L-arginine-methyl ester per h at *p*H 8.5 and 37° C.

table. The mean increases in sodium excretion were significant at each of the three doses. Mean kallikrein excretion increased at 1 ng min^{-1} though not significantly, but at 10 and 100 ng min^{-1} the increases were highly significant ($P<0.005$). The changes in excretion of sodium and kallikrein, when correlated with the logarithm of the dose of substance P infused, showed a highly significant correlation: $r=0.756$; $P<0.01$ for sodium and $r=0.735$; $P<0.01$ for kallikrein.

The data on sodium excretion place substance P amongst the most potent natriuretic substances so far described. Its threshold dose (around 1.0 ng min^{-1}) is less than, and its maximal effect greater than, those of the prostaglandins E_1, E_2 and A_1 found in comparable studies on dogs[21–23]. The concentration in the renal artery plasma which was natriuretic was approximately 0.1–1.0 ng ml^{-1} which is well below the limit of detection of a recent radioimmunoassay for substance P (ref. 24).

A variety of vasodilators are natriuretic when infused into the renal artery, for example bradykinin[25], lysyl-bradykinin[26], dopamine[27] and acetylcholine[28]. Substance P is also a vasodilator[29]. Since substance P stimulates natriuresis, salivary secretion and nasal secretion and all these processes are related to an increase in kallikrein activity, it is possible that the vasodilator action is dependent upon the release of kallikrein. There is strong evidence that urinary kallikrein originates from the kidney[14–16], being located principally in the cortex[30].

In view of the similarity between nervous stimulation and substance P infusion in their effects on stimulation of saliva flow and the related release of kallikrein and vasodilation in the salivary gland[5–7], the release of kallikrein from the kidney might be a direct effect of the substance P.

The effect of substance P on the kidney is a further example of the relation which has been shown between kallikrein excretion and sodium excretion[16–19] but in this case there is no associated expansion of body fluid volumes. The bradykinin released by kallikrein is known not only to be a vasodilator but also to increase vascular permeability. The natriuresis produced by the intra-renal release of kallikrein may be effected by vasodilator and permeability effects on the renal vasculature and tubules.

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Androgen-induced Sexual Differentiation of the Brain is Blocked by Inhibitors of DNA and RNA Synthesis

SEXUAL differentiation of gonadotrophin secretion in the rat is known to occur during the early postnatal period under the action of testicular androgens[1,2]. Animals of either sex if exposed to androgen during this critical period develop the typical male pattern of gonadotrophin secretion after puberty with failure of ovulation, small polyfollicular ovaries lacking corpora lutea and a constant cornified vaginal epithelium. Several studies have localised this effect to the brain[3,4] and more particularly to the anterior hypothalamus[5], but very little is yet known of the mechanism of this effect.

What is known of the action of androgens and oestrogens on their peripheral target tissues would suggest that the steroid enters the cell, is rapidly transported to the nucleus and, by an action on the genome, induces specific RNA and protein synthesis and later DNA synthesis and cell division[6–8]. Inhibitors of these processes might therefore be expected to provide some protection against the masculinising effects of androgen in the neonatal female rat. Attempts to achieve this with inhibitors of RNA and protein synthesis by Gorski and coworkers[9,10] have so far produced only partial and/or temporary inhibition of the action of a low dose of androgen (30 μg of testosterone propionate, TP). Barnea and Lindner[11] gave direct intracerebral injections of DNA and protein synthesis inhibitors but failed to achieve any protection against the effects of 100 μg of TP. We therefore tested the effects of a variety of inhibitors of DNA, RNA and protein synthesis combined with three different dosages of TP and have obtained evidence of permanent and, in some cases, almost complete protection against the masculinising effects of the androgen.

Female Wistar rats were treated when 4 d old with 30, 80 or 200 μg of TP s.c. in oil and the inhibitors were administered by the same route in divided doses together with and 6 h after the TP. The inhibitors used were: hydroxyurea (OH-U), an inhibitor of DNA synthesis with no effect on overall RNA synthesis[12]; α-amanitin (AMAN), an inhibitor of nucleoplasmic or messenger-precursor RNA synthesis[13]; actinomycin D (ACT.D), at the low doses used in this study, a selective inhibitor of nucleolar or ribosomal RNA synthesis[14]; puromycin (PURO), a general inhibitor of protein synthesis, and fluorouracil (FU) which induces errors of translation and the synthesis of non-functional proteins. Cycloheximide was also tested at several doses but found to be too toxic for use in neonatal rats (compare refs 10 and 11). The progress of masculinisation was followed by daily vaginal smears for at least 3 weeks prior to death at 80 to 110 d. Animals were assessed as acyclic if they showed at least 8 consecutive d of cornified smears. The ovaries were removed (where possible on the day of oestrus) and were weighed and examined for the presence of recent (functional) corpora lutea and the oviducts were examined for the presence of eggs.

Table 1 shows the results of a low (30 μg) dose of TP combined with the maximum dose of each inhibitor compatible with survival to adulthood. This dose of TP did not induce full masculinisation at 80 d of age; at this stage 20% of the animals still had functional corpora lutea and 30% showed irregular vaginal oestrous cycles. Nevertheless, the inhibitors OH-U and AMAN were capable of preventing this degree of masculinisation to a highly significant extent particularly in terms of ovarian weight ($P<0.01$ and <0.001 respectively). This was also true of a dose of AMAN one fifth of the maximum tolerated, although at this dose no clinical symptoms or disturbance of normal growth were observed during the neonatal period. The protein synthesis inhibitors (PURO and FU) were less effective but still provided a partial protection against this dose of TP ($P<0.05$). Only ACT.D was completely without effect on the development of masculinisation in these animals. When these doses of inhibitors were given alone they had no significant effects on vaginal cycles, incidence of ovulation, ovarian morphology or ovarian weight.

The results of a similar experiment using 80 μg of TP are shown in Table 2. With this dose of androgen 90% sterility and 95% acyclicity were achieved and the ovarian weight was reduced to 40% that of controls. Again AMAN and OH-U produced effective protection against masculinisation ($P<0.001$ in terms of ovarian weight); evidence of ovulation in the form of corpora lutea was present in 100% of rats given the higher dose of AMAN. The lower dose of AMAN still afforded clear protection against 80 μg of TP ($P<0.01$); however, PURO failed to provide significant protection against this dose ($P>0.10$). Once again ACT.D was completely without a protective effect.

In a further experiment a dose of 200 μg of TP was used and in this case 100% sterility and acyclicity were achieved (Table 3). A high degree of protection was again obtained with OH-U, the effect being highly significant in terms of all the parameters tested ($P<0.01$ and $P<0.001$). Indeed the ovaries were almost equal in weight to the controls which received the inhibitor alone. AMAN was markedly less effective against this high dose of TP but 33% of the animals showed signs of ovulation ($P<0.05$) and their ovaries were significantly heavier than the TP controls ($P<0.01$) although much lighter than those of rats treated with the inhibitor alone ($P<0.001$).

It is clear from these results that substances that block the synthesis of DNA or of nucleoplasmic RNA can protect the newborn female rat from the permanent neural organising effect of neonatal androgen. Inhibition of protein synthesis is less effective in this respect, and inhibition of ribosomal RNA synthesis alone (as produced by the low doses of actinomycin D we have used[14]) is completely ineffective. The former may be because we were unable to achieve a complete block to protein synthesis in the brain. Three hours after the dose of puromycin, inhibition

Table 1 Effect of 30 μg of TP Combined with Inhibitors of DNA, RNA and Protein Synthesis on Subsequent Vaginal Cycles and Ovarian Function

Treatment	Dose of inhibitor μg per 10 g b.w. per injection	Vaginal cycles No. acyclic/ total	%	Ovaries at 80 to 90 d No. with recent corpora/total	%	No. with ova in oviduct/total	%	Bilateral ovarian weight mg per 100 g ±s.e.m.
Control	—	0/14‡	0	14/14‡	100	13/14‡	93	42.3±1.7¶
TP 30 μg	—	7/10	70	2/10	20	1/10	10	24.5±3.2
TP+OH–U	8,000	2/7	29	6/7†	86	3/7	43	38.3±3.1‖
TP+AMAN	0.3	1/9†	11	8/9‡	89	6/9†	67	36.2±2.6§
TP+AMAN	1.5	0/6†	0	6/6‡	100	4/6†	67	38.6±1.6¶
TP+ACT.D	0.6	12/17	71	5/17	29	4/17	25	27.8±3.6
TP+PURO	300*	3/8	38	5/8	63	5/8†	63	36.4±3.9§
TP+FU	80	3/10	30	7/10†	70	6/10†	60	36.8±3.2§

Inhibitors given at 0 and 6 h after TP to 4-d-old rats (*given at 0, 5, and 10 h after TP).
† $P<0.05$.
‡ $P<0.01$ against TP alone by Fisher exact probability test.
§ $P<0.05$.
‖ $P<0.01$.
¶ $P<0.001$ against TP alone by Mann-Whitney U test.

of protein synthesis (by ^{35}S-methionine incorporation) was only 28% in the hypothalamus compared with 77% in the liver. Thus the lack of a protective effect of puromycin in these animals is still compatible with an obligatory role of protein synthesis in sexual differentiation of the brain.

The dependence of sexual differentiation on DNA synthesis, suggested by our results with hydroxyurea, has not been previously demonstrated. The failure of Barnea and Lindner[11] to achieve protection with hydroxyurea seems to conflict with this finding but, as these authors comment, very little is known of the time course of action of TP and it is quite possible that the effect may be more delayed than hitherto supposed. If this were the case our two large systemic doses of inhibitor given subcutaneously might well produce a longer lasting effect on the brain than their smaller doses given intracranially. This dependence is, however, entirely compatible with what is known of the process of hormone-induced differentiation in other tissues, notably the mammary gland[15]. It seemed important, therefore, to establish that some DNA synthesis was in fact continuing in the hypothalamus of the 4-d-old rat. We used ^{3}H-thymidine autoradiography to study this and have obtained evidence of DNA synthesis in both neuronal and glial cell nuclei throughout the brain, including the hypothalamus, at this age[16]. This is in accord with recent biochemical evidence obtained from whole brain fractionated into neuronal and glial nuclei[17]. Figure 1 illustrates a field in the anterior hypothalamic area but similar levels of labelling were observed in the medial preoptic area and the ventromedial/paraventricular region, the range being from 0.9 to 1.3% of cells labelled after 3 h exposure to ^{3}H-thymidine. The effect of TP on the frequency of thymidine labelling in this part of the brain is being investigated.

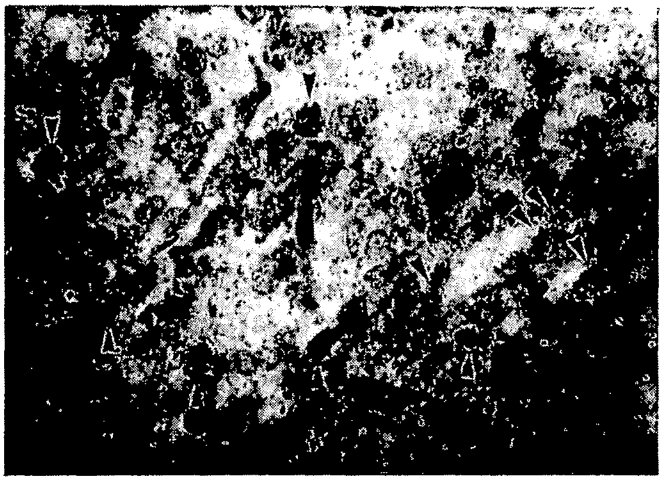

Fig. 1 Autoradiograph of parasagittal (8 μm) section from the anterior hypothalamic area (0.2 mm from the midline) of a 4-d-old female rat which received 50 μCi of ^{3}H-thymidine s.c. 3 h before death. Counter stained with nuclear fast red, exposed for 1 week at 4° C. Magnification ×390. Ten labelled nuclei are visible (arrows).

The protective action of α amanitin is equally striking since it is highly effective at a dose of one fifth of the maximum tolerated by the neonatal rat and one twentieth of that used by Tata *et al.*[13] to inhibit RNA synthesis *in vivo*. This suggests that the masculinisation process may be dependent on the synthesis of a few specific RNA molecules. It is possible that these may be histone or acidic nuclear-protein mRNA molecules the translation products of which would be necessary for the DNA synthesis that

Table 2 Effect of 80 μg of TP Combined with Inhibitors of DNA, RNA and Protein Synthesis on Subsequent Vaginal Cycles and Ovarian Function

Treatment	Dose of inhibitor μg per 10 g b.w. per injection	Vaginal cycles No. acyclic/total	%	Ovaries at 80 to 90 d No. with recent corpora/total	%	No. with ova in oviduct/total	%	Bilateral ovarian weight mg per 100 g ±s.e.m.
Control	—	0/14‡	0	14/14‡	100	13/14‡	93	42.3±1.7‖
TP 80 μg	—	20/21	95	2/21	10	0/21	0	16.8±1.1
TP+OH–U	5,000	4/13‡	31	9/13‡	69	6/13‡	46	32.7±2.6‖
TP+AMAN	0.25	2/8‡	25	6/8†	75	4/8†	50	29.4±3.4§
TP+AMAN	1.5	1/9‡	11	9/9‡	100	4/9†	44	35.5±2.6‖
TP+ACT.D	0.6	12/13	92	2/13	15	1/13	8	19.1±1.9
TP+PURO	300*	4/6	67	2/6	33	2/6	33	22.2±3.1

Inhibitors given at 0 and 6 h after TP to 4-d-old rats (*given at 0, 5 and 10 h after TP).
† $P<0.05$.
‡ $P<0.01$ against TP alone by Fisher exact probability test.
§ $P<0.01$.
‖ $P<0.001$ against TP alone by Mann-Whitney U test.

Table 3 Effect of 200 μg of TP Combined with Inhibitors of DNA and RNA Synthesis on Subsequent Vaginal Cycles and Ovarian Function

Treatment	Dose of inhibitor μg per 10 g b.w. per injection	Vaginal cycles No. acyclic/total	%	Ovaries at 95 to 110 d No. with recent corpora/total	%	No. with ova in oviduct/total	%	Bilateral ovarian weight mg per 100 g ±s.e.m.
Control	—	1/16†	6	15/16†	94	13/16†	81	33.6±1.5‖
TP 200 μg	—	13/13	100	0/13	0	0/13	0	16.1±1.5
TP+OH–U	4,000	2/11†	18	8/11†	73	6/11†	55	35.0±2.4‖
TP+AMAN	1.5	11/15	73	5/15*	33	3/15	20	23.8±2.0‡
OH-U control	4,000	0/8†	0	8/8†	100	6/8†	75	36.6±1.9‖
AMAN control	1.5	0/13†	0	13/13†	100	11/13†	85	35.4±1.2‖

Inhibitors given at 0 and 6 h after TP to 4-d-old female rats.
* $P<0.05$.
† $P<0.01$ against TP alone by Fisher's exact probability test.
‡ $P<0.01$.
‖ $P<0.001$ against TP alone by Mann-Whitney U test.

might follow. Hydroxyurea is known to block the synthesis of these specific mRNAs selectively[18].

Although the precise mechanism of sexual differentiation remains obscure, it is apparent from these results that DNA, RNA and probably also protein synthesis is involved at an early stage; in this respect the process is closely analogous to hormone-induced differentiation in other tissues of the body, for example, in the mammary gland[15] or the erythropoietic system[19].

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Dissociation of Stimulatory and Synthetic Phases in the Induction of Tyrosine Hydroxylase

RESERPINE treatment causes an increase in the activity of tyrosine hydroxylase (T-OH) in the superior cervical ganglion of the rat. A single systemic injection of the drug produces a rise in enzyme activity that reaches a maximum 48–72 h later[1]. No change in T-OH is seen if the preganglionic cholinergic tract is cut at the time of reserpine administration or if animals are pretreated with the ganglion blocking agents pempidine or chlorisondamine[2,3]. In the cat, direct electrical recording from the preganglionic cervical sympathetic trunk has revealed increased neuronal firing after reserpine administration[4]. These findings suggest that the rise in T-OH activity elicited by reserpine is mediated trans-synaptically through an increase in ganglionic transmission.

Little is known about the sequence of events that occurs between the injection of reserpine and the increase in T-OH activity. We have used a combination of *in vivo* administration of reserpine with subsequent *in vitro* maintenance of superior cervical ganglia to achieve a temporal dissociation of the trans-synaptic stimulation of the ganglia from the actual increase in T-OH activity. We have also used this system to assess the importance of continuing protein synthesis in the ganglion for the development of this increase in enzyme activity.

Adult male Wistar rats (250–350 g) were injected subcutaneously with 5 mg kg^{-1} reserpine (British Drug Houses, Poole) in 20% ascorbic acid or with the vehicle alone. Superior cervical ganglia were removed 24 or 72 h later and the ganglion sheaths removed. The ganglia were then either frozen at −20° C or placed in organ culture. The organ culture system involved placing ganglia on Millipore filters supported on metal grids in Falcon organ culture dishes (Becton, Dickinson and Co., Wembley, Middlesex). Eagle's minimum essential medium was supplemented with glucose (to 600 mg%), neonatal calf serum (10% v/v) and penicillin and streptomycin (100 IU ml^{-1}). The dishes were incubated at 37° C in an incubator perfused with humidified 95% O_2 and 5% CO_2 for 48 h. After incubation, ganglia were washed thoroughly in basic salt solution and stored at −20° C until assayed. Individual ganglia were homogenised in 20 or 60 µl of 5 mM Tris buffer, *p*H 7.4, containing 0.1% Triton X-100. Tyrosine hydroxylase activity was assayed in 10 µl aliquots using L-tyrosine (2,3,-^{3}H-side chain) as substrate at a concentration of 8 µM (ref. 5). Protein content was determined using the method of Lowry[6].

Table 1 Tyrosine Hydroxylase Activity in Rat Superior Cervical Ganglia Following Reserpine Treatment

	T-OH activity as % *in vivo* controls
In vivo controls	100±11
Reserpine 24 h	102± 6
Reserpine 72 h	186±10
Culture controls	86± 4
Reserpine culture	158± 9 (185±10% of culture control)

The tyrosine hydroxylase activity in the five experimental groups is expressed as a percentage of the *in vivo* controls. Means and s.e.m. are based on groups of thirty ganglia (pooled results from five experiments). The tyrosine hydroxylase activity in the *in vivo* controls was 350 pmol dopa per h per mg protein. 'Reserpine 24 h' and 'reserpine 72 h' refer to values obtained from ganglia removed from animals 24 h and 72 h after administration of reserpine (5 mg kg^{-1}, subcutaneously). 'Culture' results were from ganglia maintained in organ culture for 48 h; 'Reserpine culture' results from ganglia explanted 24 h after administration of reserpine.

Five experimental groups were studied: '*in vivo* controls' (ganglia from rats injected with vehicle alone 24 or 72 h previously), 'reserpine 24 h' (ganglia from rats injected 24 h previously with reserpine), 'reserpine 72 h' (ganglia from rats injected 72 h previously with reserpine), 'culture controls' (ganglia excised from rats injected with vehicle alone 24 h previously and subsequently maintained for 48 h in organ culture), 'reserpine culture' (ganglia from rats injected 24 h previously with reserpine and subsequently maintained for 48 h in organ culture). The *in vivo* control level of T-OH activity was 350 pmol dopa per h per mg protein. No change in T-OH activity was observed in the reserpine 24 h group when compared with the *in vivo* controls. The reserpine 72 h ganglia, however, showed an 86% increase (Table 1). Over the 48 h culture period, the T-OH activity in the culture control ganglia fell on average to 86% of the *in vivo* controls. On the other hand, the reserpine culture group showed a 58% rise in T-OH activity relative to *in vivo* control and an 85% rise relative to 'culture control'. Thus ganglia removed from the animal before any increase in T-OH activity had developed showed an increase in the enzyme in culture comparable with that which would have been achieved *in vivo*. These findings

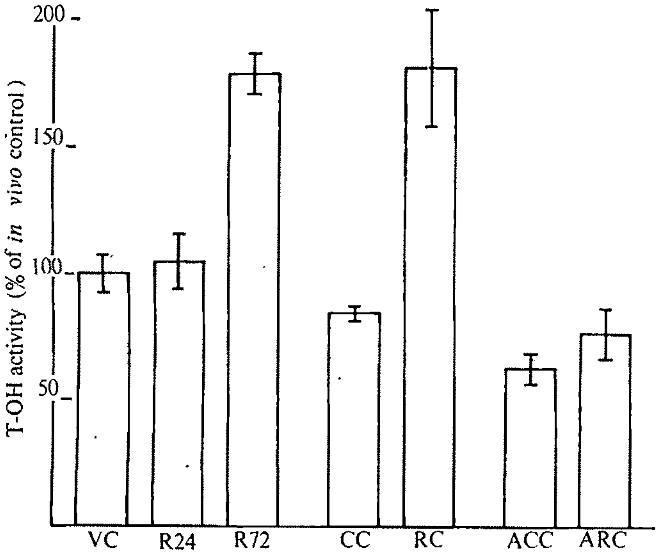

Fig. 1 Effect of anisomycin on the increase in tyrosine hydroxylase activity following reserpine treatment. The means ± s.e.m. are expressed as percentages of *in vivo* control values. Six ganglia were assayed in each group. The groups were: VC = *in vivo* controls, R24 = reserpine 24 h, R72 = reserpine 72 h, CC = culture control, RC = reserpine culture, ACC = anisomycin (2 μg ml^{-1}) culture control, ARC = anisomycin (2 μg ml^{-1}) reserpine culture. Mean tyrosine hydroxylase activity of *in vivo* control group was 350 pmol dopa h^{-1} per mg protein. For details see Table 1 and text.

suggest that any stimulation of the ganglion in the intact animal beyond that achieved within 24 h of a single injection of reserpine is unimportant to the expression of increased T-OH activity.

To investigate the role of protein synthesis in the development of increased T-OH activity one group of reserpine culture ganglia was maintained for 48 h in the presence of anisomycin, a specific blocker of peptide bond formation[7], at a concentration of 2 μg ml^{-1} in the culture medium. The enzyme activity was compared with that of a similar group of reserpine culture ganglia maintained for 48 h in normal medium. The T-OH rise normally seen after reserpine was completely abolished in the anisomycin-treated group (Fig. 1). This finding that inhibition of protein synthesis in the isolated ganglion abolished the reserpine-stimulated rise in T-OH activity confirms earlier interpretations of the effects of cycloheximide administered to the whole animal[8]. Our results clearly demonstrate that an important site of this effect of protein synthesis inhibitors is the ganglion itself.

By removing the ganglion from the animal and maintaining it in culture we have been able to show that after a period of stimulation the adrenergic cell bodies can produce an increase in T-OH activity when isolated from the neural and humoral changes produced *in vivo* by reserpine. Furthermore, this increase in enzyme activity is dependent on protein synthesis.

A recent study using actinomycin D suggests that the transcriptional phase of the rise in T-OH activity of rat stellate ganglia evoked by a 2 h exposure to stress is complete 24 h later[9]. Other workers have found that 12 h after insulin treatment actinomycin D can no longer prevent the insulin-induced rise in adrenal T-OH activity[10]. The time course of events following reserpine treatment has still to be elucidated.

The system we have described should provide a useful means of investigating this and other questions concerning the cellular mechanisms by which neurones respond to trans-synaptic stimulation.

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Preparation of Frozen Hydrated Tissue Sections for X-ray Microanalysis in the Scannning Electron Microscope

THE technique of X-ray microanalysis, using a finely focused beam of electrons to excite characteristic X rays from the elements present in a specimen, is proving to be a powerful tool in the investigation of biological systems. Its main limitation has been that, in common with all electron-optical methods, the specimen must first be stabilised by removal or immobilisation of cell and interstitial water. Conventional chemical fixation techniques give excellent structural preservation, but also cause gross permeability changes in cell membranes and, together with subsequent dehydration, lead to substantial loss or redistribution of free electrolytes. Freeze drying suffers from a similar limitation, in that removal of water must inevitably redistribute electrolytes which move to the remaining structures such as cellular membranes.

To study the distribution of water-soluble ions in biological systems, we have therefore developed a technique for immobilisation of water and solutes by rapid freezing, followed by sectioning and examination of the tissue while still in the frozen, hydrated state, that is with cellular and interstitial water retained as ice at a low temperature. A similar approach is also being adopted by Gehring *et al.*[1] and by Hutchinson (private communication).

Full details of the preparative procedure are given elsewhere in papers on technique[2–5]: the principal purpose of this communication is to describe our first successful results. The specimens were salivary glands isolated from 2 to 3-d-old larvae of the blowfly (*Calliphora*). These were mounted in either insect blood or a balanced medium[6] on an aluminium chuck and quench frozen in liquid fluorocarbon at −150° C (ref. 3). The chuck was transferred to a Slee retracting microtome in a large cryostat chamber at −85° C, and sections 1 to 2 μm thick were cut using a steel knife. Sections were picked up on aluminised nylon support films[5], stretched over aluminium collars designed to fit into a copper specimen holder for examination and microanalysis. The collars with

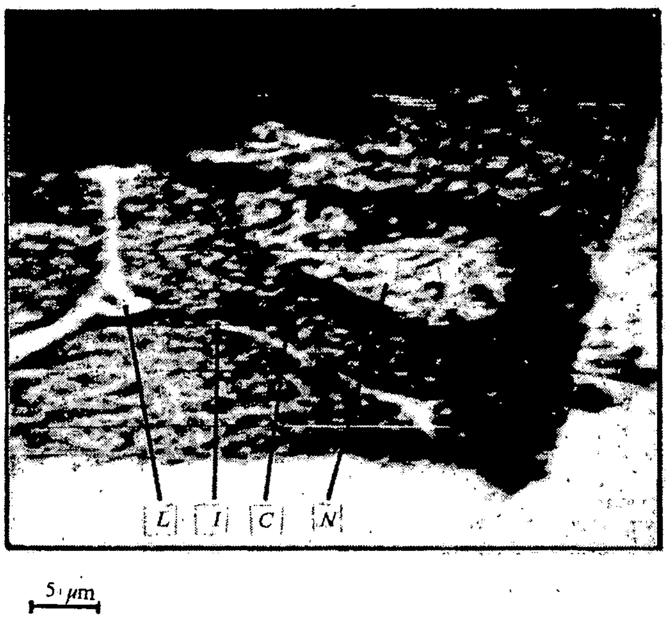

Fig. 1 Scanning transmission electron micrograph showing a frozen, hydrated transverse section of a blowfly larval salivary gland. *C*, Cytoplasm; *N*, nucleus; *L*, lumen; *I*, intercellular cleft.

sections attached were immediately immersed in liquid nitrogen, where they could be stored before fitting into the specimen-holder. Contamination of microtome and sections by surface ice was prevented by careful exclusion of atmospheric air during sectioning and transfer: the cryostat chamber was divided by a vertical polyethylene curtain separating the manipulation area from the microtome, and all manipulations were carried out either in the cryostat or under liquid nitrogen.

For critical work using diffracting spectrometers and high probe currents, it would be desirable to coat the mounted specimens, still maintained at a low temperature, with a further layer of evaporated metal to improve their stability and conductivity. In the experiments described here, where an energy-selective spectrometer and moderate beam currents were used, this complication was found not to be necessary and was omitted. The mounted sections, under liquid nitrogen, were enclosed in a protective metal cap[5] and transferred directly to the pre-cooled stage of a Stereoscan S4 scanning electron microscope (Cambridge Scientific Instruments Ltd).

The cold-stage consists of a standard Cambridge Instruments Series 200 microanalysis stage with hot-cold module, which we have modified to allow the examination of thin specimens in the transmission mode[3]. The detector for transmitted electrons is a silicon solar-cell (Centralab, California) approximately 2 mm in diameter, mounted about 40 mm below the specimen and coupled through a pre-amplifier to the video system of the Stereoscan. Our modifications have also increased the working distance from the specimen to the final lens of the microscope to about 18 mm. This allows us to insert the 'nose' of a lithium-drifted silicon X-ray detector (active area 25 mm^2; Quartz et Silice, Paris) close to the horizontally mounted specimen.

Figure 1 shows the scanning transmission electron image of a transverse section of a salivary gland, taken with an operating potential of 30 kV. Resolution at this potential is severely limited by scattering of electrons in the specimen, which is relatively thick compared with those normally used for transmission electron miscroscopy. Contrast is good, however, and the major cellular features are clearly identifiable.

X-ray microanalysis was carried out by advancing the detector as close as possible to the specimen (distance 10 to 15 mm) and allowing the electron beam to scan an area of specimen 5 μm square. Figure 2 shows X-ray spectra from areas in the nucleus, cytoplasm and lumen of the gland, each obtained with a 30 kV, 4 nA probe over an irradiation period of 100 s. The stage temperature was −160° C. The spectra show clearly the presence of a large concentration of potassium in the cell, but not in the lumen. Phosphorus and sulphur are also present in the cell; signals from copper, iron and silicon are background resulting from scattered electrons, and that from aluminium is generated mainly in the supporting film. Although these spectra do not by themselves permit accurate quantitative microanalysis, they are sufficient to show that ions are retained in the specimen during preparation. By using stable inorganic standards and the higher X-ray resolution obtainable with diffracting spectrometers, fully quantitative measurements will be possible.

Stability of the specimen under the beam was investigated by using the X-ray continuum intensity as a measure of the total mass in the irradiated portion of the specimen[7]. In Fig. 3, *A* shows local mass as a function of time in conditions similar to those used for microanalysis. The curve is similar to those obtained by Hall and Gupta[8] from cooled, freeze-dried tissue sections, showing only a very slow loss of material due to chemical and thermal damage by the high-energy electron beam. The early loss of mass which is normally found with room-temperature specimens was found in the present experiments only after the specimen had been warmed (Fig. 3*B*). It is thus very unlikely that any substantial loss or redistribution of inorganic ions could take place as a result of irradiation.

Apart from a brief period at about −80° C during sectioning, the temperature of the specimen during preparation and transfer never rose above −120° C, and it was always protected from exposure to the air. It is therefore most unlikely that any melting or drying would occur. Direct evidence that the specimen as examined was indeed hydrated

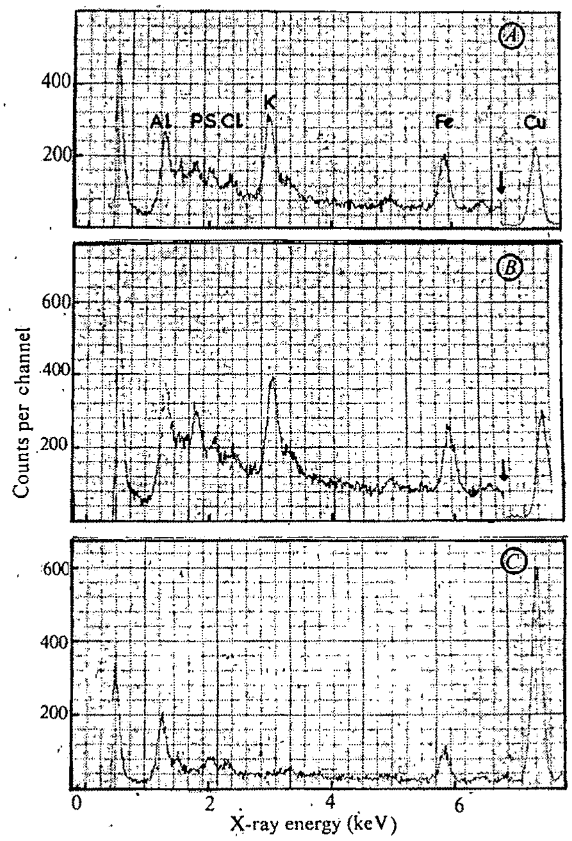

Fig. 2 X-ray spectra from the specimen of Fig. 1. *A*, Nucleus; *B*, cytoplasm; *C*, lumen. In *A* and *B* the vertical scale was reduced by a factor of 5 at the arrowed point, to keep the copper peak on scale.

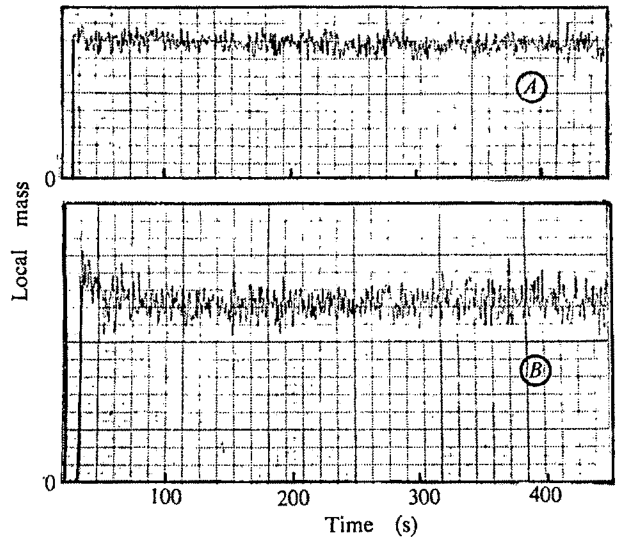

Fig. 3 Local mass (arbitrary units) against time during irradiation of the specimen. *A*, At −160° C; *B*, at room temperature, after sublimation of ice from the specimen.

was obtained by allowing the stage temperature to rise slowly, while continuing to watch the scanning transmission and secondary-electron 'reflection' images. At about −75° C, a transient rise in vacuum pressure indicated the sublimation of ice from stage and specimen, and was accompanied by an increase in contrast of the transmission image. The secondary-electron image, which had previously been almost featureless, now showed recognisable outlines of the cells (this is consistent with the results of Gehring *et al.*[1], who found it necessary to etch the surface of a frozen bulk specimen, in order to obtain a useful secondary-electron image).

We believe that Fig. 1 is the first scanning transmission electron image to be obtained from a frozen, hydrated tissue section, with firm evidence that the specimen did not suffer dehydration during preparation, transfer or examination. Although these results are of a preliminary nature, and do not in themselves significantly advance our knowledge of cells and tissues, we believe that they demonstrate the feasibility of a technique which is capable of providing answers to numerous biological problems. From the present results to the analysis of ion distributions as they exist in living tissues, on a scale of 1 μm or less, is a step involving no more than the use of more specialised instrumentation already commercially available.

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Number of Ouabain-binding Sites in Fibroblasts from Normal Subjects and Patients with Cystic Fibrosis

One of the characteristics of cystic fibrosis (CF), an autosomal recessive disease, is a defect in the net sodium transport in the eccrine glands resulting in an increased concentration of sodium in the sweat[1]. This defect has yet to be characterised as an extrinsic and/or intrinsic phenomenon. Mangos and McSherry[2] demonstrated an unidentified factor in the sweat from CF patients which inhibited sodium reabsorption in the rat parotid duct after retrograde perfusion. The relationship of the sweat factor to factors observed in saliva[3] and sera[4,5] of CF patients has yet to be elucidated.

Although there is no direct evidence for an intrinsic defect within the gland, studies of erythrocytes indicate that a defect could exist. Balfe *et al.*[6] reported abnormal sodium transport in erythrocytes from CF patients; Lapey and Gardner[7] demonstrated a decrease in the pump II component of sodium efflux in male CF patients; and Horton *et al.*[8] reported a decrease in calcium-activated ATPase in CF erythrocytes. Fitzpatrick *et al.*[9] observed an altered electrophoretic pattern of CF erythrocyte membranes, which they interpreted as an altered calcium-binding component in the membrane. However, Hadden *et al.*[10] could not demonstrate significant differences in the sodium-potassium ion exchange and in the calcium efflux pump of CF and normal erythrocytes when comparisons were matched for age and sex.

Recently, binding of ^{3}H-ouabain to the cell membrane has been used to determine the number of ouabain-binding sites per cell in red blood cells[11–13], in cultured Girardi heart cells[14], and in HeLa cells[11,15,16]. These studies demonstrated a direct correlation between the number of ouabain-binding sites per cell (at low ouabain concentrations) and the Na^{+}–K^{+} ATPase activity.

As Hadden *et al.*[10] have said, the differences observed between CF and normal erythrocytes are difficult to interpret because the CF cells may be affected by the anoxia, infection, malabsorption, liver disease and vitamin or antibiotic therapy seen in CF patients. Recently, Fletcher and Lin[18] found no differences in the Na^{+}–K^{+} ATPase activity, measured in a heavy particulate fraction from fibroblasts of normal subjects and CF patients after cell homogenisation. Because of possible complications in the erythrocytes from CF patients, and since the activity observed in a cell homogenate may not express the situation in the intact cell, we have looked for differences in the binding of ^{3}H-ouabain in cultured normal and CF human diploid fibroblasts.

Human fibroblast cell strains were established from skin biopsy explants. The fibroblasts, after outgrowth, were frozen and stored in liquid nitrogen. The three normal and three CF fibroblast cell strains used had undergone less than ten subcultures since the establishment of the cell strain from the biopsy. The fibroblasts were cultured in F-12 (Gibco) containing 15% foetal calf serum (Gibco) and 100 U ml^{-1} of penicillin and 100 μg ml^{-1} streptomycin.

The number of binding sites for CF and normal human diploid fibroblasts was determined on cells in log phase (Fig. 1). The initial determination was made 24 h after inoculation of the cultures and additional determinations were carried out on days 2 and 4. As Figure 2 shows, we observed no appreciable differences in the number of ouabain-binding sites between normal and CF cell strains. The mean number of sites per cell for the normal fibroblasts was $7.0 \times 10^6 \pm 2.3$ s.d. and for the CF fibroblasts $8.3 \times 10^6 \pm 1.5$ s.d.

Boardman *et al.*[16] found that HeLa cells, after exposure to sublethal levels of ouabain for 24 h, had an increased number of ouabain-binding sites per cell and a concomitant increase in their Na^+–K^+ ATPase activity. Thus the cells compensated for the decreased Na^+–K^+ ATPase activity caused by ouabain inhibition of the enzyme. Since there was no apparent change in the number of binding sites during logarithmic growth of the CF cell strains (Fig. 2), it seems that the ciliary dyskinetic factor which accumulates in the media of CF fibroblasts[17] does not interfere with their Na^+–K^+ ATPase activity. Furthermore, the constancy in the number of ouabain-binding sites in CF fibroblasts indicates that the factor does not appreciably affect the permeability of the plasma membrane; that is, an increased number of binding sites would be expected[16] to maintain the normal intracellular ionic environment if the permeability of the membrane had increased.

Our observations also agree with the studies of Fletcher and Lin[18], who observed no differences in the membrane ATPase activity and its response to inhibition by ouabain and ethacrynic acid in CF and normal fibroblasts.

We were also unable to observe any differences in the kinetics of ouabain-binding between normal and CF fibroblasts in K^+-free phosphate-buffered saline solution, and the results were essentially the same as those seen in HeLa cells[15,16]; that is, saturation was achieved within 30 min at 37° C (2.0×10^{-7} mol l^{-1} ouabain) and remained constant for more than 1 h.

The studies on the Na^+–K^+ ATPase in CF fibroblasts, measured in a heavy particulate fraction from a cell homogenate, have indicated no observable differences in the specific activity of the Na^+–K^+ ATPase nor any alteration in the inhibitory pattern of the Na^+–K^+ ATPase *per se*[18]. Using intact cells, we observed no alterations in the kinetics of binding of ouabain nor any alteration in the total number of binding sites. These data indicate that the Na^+–K^+ ATPase functions normally in the CF fibroblast.

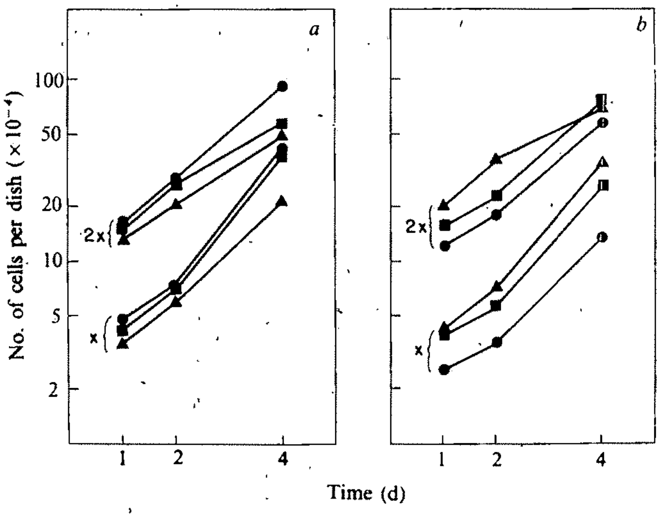

Fig. 1 The cell strains were grown to confluency and trypsinised. The resultant suspensions of cells were inoculated into P-60 Falcon tissue culture dishes at an approximate cell concentration of 4 (X) and 8 (2X) × 10^4 cells per dish. The cells were cultured in F-12 (Gibco) containing 15% foetal calf serum. At day 1, 2, and 4, 3 P-60 Falcon plastic dishes for each cell strain at each cell density were washed with saline, trypsinised and the total cell number per dish was determined. Results shown are the mean of the three determinations. *a*, Normal fibroblast: strains were (▲), No. 17; (■), No. 18; (●), No. 31. *b*, CF fibroblast: (▲), No. 2; (■) No. 4; (●), No. 24.

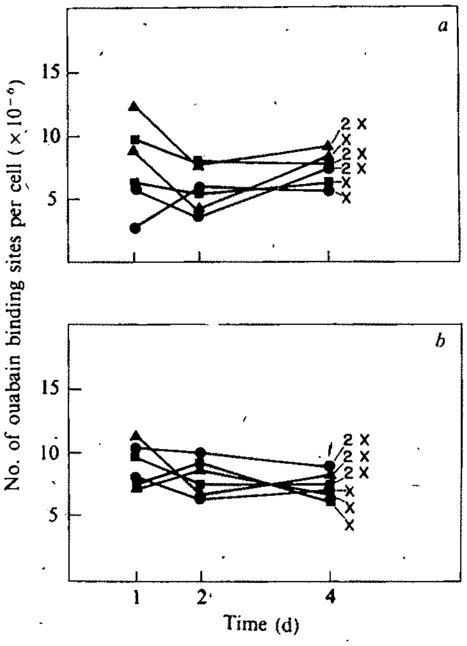

Fig. 2 The cell culture media were removed and the cells were washed with a potassium-free phosphate-buffered saline, *p*H 7.4, 37° C, which contained 0.01% glucose (PBS-g). The ^{3}H-ouabain (New England Nuclear Corp.) was added in a PBS-g solution (37° C), and the cells were incubated for 30 min. The ^{3}H-ouabain solution was removed, and the cells were washed four times with excess quantities of PBS-g at 0° C. The final wash was removed, the cells were trypsinised, and the entire contents of the culture dish were transferred to counting vials for liquid scintillation counting. The ouabain concentration used was 2.08×10^{-7} mol l^{-1}, and the specific activity was 2.3×10^7 mol per d.p.m. Each point corresponds to the mean of three determinations. X and 2X correspond to the inoculum cell concentration. The cell number per dish at the time of each determination is seen in Fig. 1. *a*, Normal fibroblast: strains were (▲), No. 17; (■), No. 18; (●), No. 31. *b*, CF fibroblast: (▲), No. 2; (■), No. 4; (●), No. 24.

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Resistance to Marek's Disease in Immunologically Deficient Chickens

A CELL-ASSOCIATED herpesvirus has been shown to induce Marek's disease (MD), a naturally occurring malignant neoplasm of chickens. The mechanism of tumorigenesis or the factors that mediate natural or acquired resistance to MD are not well understood. Information on these aspects is needed for a better understanding of the basic mechanisms of this and possibly other herpesvirus-induced neoplasms.

Inherited resistance of chickens to MD has been known for many years[1], and several inbred lines selected for natural resistance are available. We observed that chickens of one such line, designated line 6, when exposed to MD virus (MDV), developed persistent viraemia and antibody but no clinical disease[2]. The mechanism of resistance is unknown, but in confirmation of Calnek's observations on another genetically resistant line[3], we found that line 6 chickens produced a better virus neutralising (VN) antibody response than simultaneously inoculated genetically susceptible chickens. Thus, it was postulated that genetic resistance to this disease has an immunological basis and may depend on the ability of chickens to produce VN antibody. In testing this postulate I reasoned that since antibody synthesis is a function of cells derived from the bursa of Fabricius[4], response to MDV inoculation of bursectomised genetically resistant chickens should provide proof of the involvement of humoral antibody in this type of resistance.

Two experiments were conducted. The White Leghorn chickens used had maternal MD antibody at hatching. Chickens of line 6 known to be resistant to clinical MD[2,5] were either surgically bursectomised on the seventeenth day of embryonic life or sham operated as described by Van Alten *et al.*[6]. Surgical bursectomy at or before this stage eliminates or severely impairs the ability of chickens to synthesise both IgM and IgG[7]. One week after hatching, bursectomised, sham operated and untreated chickens of line 6 and line 7 hatchmates (highly susceptible line) were either inoculated with the JM 19 clone[8] of MDV (1.8×10^5 and 1.0×10^5 PFU per chicken in experiments 1 and 2 respectively), or left as uninoculated controls. In both experiments 33–44% of hatched chickens from bursectomised and sham operated groups died of herniated gut before virus inoculation. Each group was maintained in a separate Horsfall–Bauer isolator under negative pressure and was given regular feed and medicated water (Floxaid, 2 ounces per 50 gallons of drinking water, Merck). At 4 and 6 weeks old, each bursectomised and sham operated chicken was inoculated intramuscularly with 10^9 sheep erythrocytes suspended in 0.5 ml of phosphate-buffered saline. The inoculum was deposited at either a single site or three different sites in the same bird.

In both experiments, 8 weeks after inoculation, all surviving chickens were bled by cardiac puncture and then killed. Certain groups were tested for levels of viraemia by inoculating white blood cells from individual chickens onto secondary cultures of duck embryo fibroblasts[9] and counting foci of cytopathic effects 10–14 d later. Plasma or serum samples were tested by some or all of the following tests: agar gel precipitin (AGP)[10], immunofluorescent (IF)[11] and VN tests for anti-MDV antibody[2]; haemagglutination (HA) test for anti-sheep erythrocyte agglutinins[12]; radial diffusion test[13–15] for values of IgM and IgG (conducted by Dr M. D. Cooper). All chickens were examined grossly and the following sections were taken for histological examination: both vagi, right and left sciatic, and right and left brachial plexuses, gonads, spleen and bursa. If bursal remnants could not be detected grossly, a small area at the usual location of the bursa was sectioned. All sections were stained with haematoxylin and eosin and additional sections of spleen were stained with methyl green-pyronin also[16].

Since the design and results of both experiments were similar, data were pooled and are given in Tables 1 and 2. Of sixteen survivors in the bursectomised group, eleven had no detectable bursal remnant. These eleven were all deficient in bursa functions, that is, they produced no anti-sheep erythrocyte agglutinins, they had no detectable germinal centres in spleen and their sera lacked detectable levels of IgM and IgG (Table 1). The five chickens with bursal remnants and all sham operated chickens had well developed bursa-dependent functions (Table 1). The response of bursectomised, sham operated and untreated line 6 chickens to MDV is given in Table 2. Only the chickens that lacked gross and microscopic evidence of bursa and bursa-dependent functions are included in the bursectomised group. The incidence of MD in genetically susceptible line 7 chickens was high (approximately 90%), indicating that MDV inoculum was highly pathogenic. However, clinical MD did not develop in bursectomised, sham operated and untreated chickens of genetically resistant line 6. Bursectomised chickens lacked AGP, IF and VN antibody against MDV in contrast to the high incidence of antibody detectable by all three tests in sham operated and untreated groups. Four of eleven bursa-deficient chickens had viraemia, evidence that chickens in this group were infected with MDV. In one experiment, five of thirteen uninoculated control chickens of line 6 had AGP antibody indicating inadvertent exposure to MDV. However, infection in this control group apparently did not affect the outcome of this study because in the second experiment where control chickens remained free of MD the results were similar.

In this study, although a limited number of bursectomised chickens were available, the results were considered valid because a complete morphological and functional depletion of bursa was achieved without endangering the functions of other tissues or systems. Also, since line 6 chickens are highly resistant to MD, any variation in their response would have been detectable even among the small numbers used.

These experiments demonstrated that bursectomised chickens of line 6, although devoid of immunoglobulins, maintained their resistance to MD. Unpublished studies of Payne and Rennie (quoted by Payne[17]) reportedly produced similar results. Although these studies strongly suggest that the ability to synthesise humoral antibody was not a critical factor

Table 1 Bursa-dependent Functions in Bursectomised and Sham Operated Line 6 Chickens

Embryonic treatment	Bursal remnant*	No. of chickens	Germinal centres per spleen section†		HA titre ($\log_2$)‡		mg% IgM in serum		mg % IgG in serum	
			Mean	Range	Mean	Range	Mean	Range	Mean	Range
Bursectomy	Absent	11	0.0	—	0.0	—	0.0	—	0.0	—
Bursectomy	Present	5	6.15	2–11	6.8	3–≥9	29.4	13.5–44.7	48.9	26.0–95
Sham operated	Present	18	15.45	3–36	7.2	5–≥9	27.6	13.5–55.1	109.3	0.0–201.0

Data were pooled from two experiments.
* Detectable by gross and (or) microscopic examination.
† Spleen was sectioned approximately through the middle and all germinal centres present in the entire cross section were counted.
‡ Sheep erythrocyte agglutinins.

Table 2 Response of Bursectomised, Sham Operated and Untreated Chickens of Line 6 to Marek's Disease Virus (MDV) Inoculation

No. of chickens	Embryonal treatment	Inoculum	Viraemia		MD-Antibody†				MD		
			+/ tested	Mean* titre	AGP +/ tested	VN +/ tested	IF +/ tested	Mean titre	No. died	No. with lesions‡ at termination	Total MD
11	Bursectomised	MDV§	4/11	2.6	0/11	0/11	0/11	0	0	0	0/11
18	Sham operated	MDV	2/18	0.25	12/18	18/18	18/18	≥142	0	0	0/18
29	None	MDV	NT	NT	28/28	NT	28/28	≥134	0	0	0/28
28	None	None	NT	NT	5/28¶	NT	NT	NT	0	0	0/28

Data are pooled from two experiments. NT, not tested.

* PFU per 10^7 white blood cells.

† AGP test was done on undiluted sera. For VN test, all samples were diluted 1 : 20. The IF antibody titre corresponds to the reciprocal of highest serum dilution giving a positive reaction (1 : 160 was the highest dilution tested).

‡ Gross or microscopic.

§ The MDV inoculum induced MD in approximately 90% of simultaneously inoculated line 7 chickens.

¶ Presence of AGP antibody indicates inadvertent exposure to MDV of this group in one experiment.

in resistance to MD, the following points must be considered before definite conclusions are reached.

(1) It can be postulated that bursa-derived (B) cells act as target cells for lymphoma formation by MDV and that embryonic bursectomy eliminated all available B cells from the host. Indeed, in lymphoid leukosis, another haematopoietic neoplasm of chickens, neoplastic transformation originates in the cells of the bursa of Fabricius and bursectomy during early stages of infection prevents clinical disease[18–20]. Thus, by this reasoning, lack of MD in bursectomised chickens was due basically to the lack of target cells. Although conclusive proof is lacking, available data suggest that the target cell in MD is not a B cell. The most direct evidence comes from the observation that MD lymphomas can be induced in susceptible, bursectomised, X-irradiated, agammaglobulinaemic chickens[21]. However, in this study, bursectomy and X-irradiation were performed at hatching, and since bursa-derived cells peripheralise during embryonic stages, it is possible but unlikely[22] that some B cells were available at the time of virus inoculation. Conclusions of Foster and Moll that bursectomy decreased MD[23] were not confirmed by others who found bursectomy either to have no effect[21,24] or to enhance the severity of MD in susceptible chickens[25,26]. Also, Hudson and Payne[27] and Rouse *et al.*[28] found that in MD lymphoma, most lymphoid cells (80% or more) were of thymic origin.

(2) Line 6 chickens used in this study had maternal antibody against MDV at hatching and bursectomy presumably did not influence the protective effect this antibody may have had on their response. Although this argument cannot be settled until after a similar study of line 6 chickens without maternal antibody, it seems unlikely that maternal antibody alone was responsible for the resistance in these chickens. Studies where the protective effect of maternal antibody was quantitated[29] showed that susceptible chickens with maternal antibody could neutralise approximately 1 $\log_{10}$ of cell-associated virus. Since in my study, line 6 chickens were challenged with 10^5 or more PFU of pathogenic MDV, and a dose of 1 PFU or less can induce neoplastic response in the susceptible chicken[5], it seems unlikely that maternal antibody seriously affected the outcome of this study. Nonetheless, in view of Calnek's observation[30] that maternal antibody was important in the expression of early resistance of N line chickens, the role of maternal antibody in MD needs further clarification.

The mechanism of resistance to MD is not clear. Suggestions include (*a*) selective ability of resistant chickens to produce VN antibodies[2,3]; (*b*) cell-mediated immunity[17]; (*c*) increased production in resistant chickens of non-specific inhibitors such as interferon[31]. Although these possibilities may not be mutually exclusive, the study reported here clearly demonstrates that ability to produce antibody is not required in the expression of resistance to MD.

I thank Dr M. D. Cooper, University of Alabama, for demonstrating embryonal bursectomy and for conducting tests for IgG and IgM. I also thank Drs H. G. Purchase and R. L. Witter for advice and assistance. Chickens were made available through Mr H. A. Stone. Mrs Evelyn Young and Mrs Karen Juntikka provided technical assistance.

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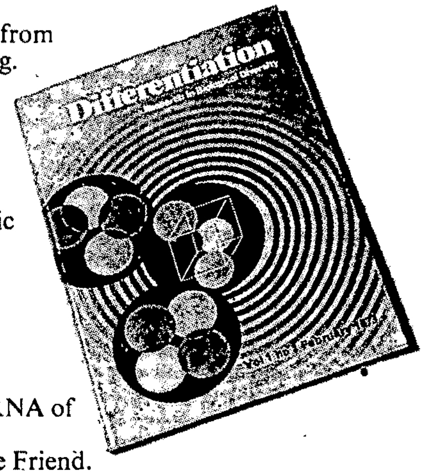

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Nutrition reviewed

World Review of Nutrition and Dietetics. Edited by Geoffrey H. Bourne. Vol. 17 pp. xii+318; vol. 18 pp. 336. (Karger: Basel, London and New York, September and October 1973.) Vol. 17: Sfr. 144; £20.90; $57.75; vol. 18: Sfr. 165; £23.95; $59.15.

THE two latest volumes of this ambitiously named series contain three quite different types of contributions; one group is composed of technical reports of particular aspects of biochemical research; the second, consisting of two papers, contains accounts of the changes in food supply and their consequent effect on nutrition in different communities; while the third, again made up of two contrasting pieces of writing, deals with the very different philosophical outlooks with which workers of different origins have come, after a generation of hard experience, to view the practice of applying nutrition and dietetics to the problems of diverse communities. In spite of the wealth of scholarship deployed in the 654 pages of the two books, it would be a naive purchaser who, after paying the combined price of £44.85, considered that he had received his money's worth.

Some of the research reviews are excellent. E. B. Olsen and H. F. De Luca contribute an admirable paper to volume 17 on the recent advances in understanding of the metabolism and mechanism of action of vitamin D. That by I. Szelenyi in the same volume on magnesium and its significance to cardiovascular and gastro-intestinal disorders is also stimulating, as a review article should be. For anyone who wants to know all about the use of molasses for feeding cattle or about the use of citrus fruits for feeding people (apparently more citrus fruit is eaten than any other kind), T. R. Preston has an exhaustive paper in volume 17 and J. Kefford an equally comprehensive one in volume 18. C. G. King contributes a good review covering the biological synthesis of ascorbic acid which possesses the added advantage of being succinct; the paper by J. T. Snook on protein digestion is equally good.

The two articles, one by S. C. Hsu describing what has happened to nutrition in Taiwan between 1945 and 1970, in volume 17, and the other by E. Ferber reviewing conditions in Yugoslavia between 1953 and 1963, in volume 18, take us into a different area of nutrition and dietetics. The minutiae of the biochemistry of vitamins, of magnesium and water-borne goitrogens, about which E. Gaitan has an article in volume 17, are some of the essential bricks from which our knowledge of nutrition is built up. What use can be made of the edifice of scholarship is demonstrated in the narrative of communities of real people. It is easy to see that the work in Taiwan was influenced by American thinking. The tables of figures are all there, the dietary surveys, anthropometric data and plans to regulate the labelling of food and provide nutrition education for the public. In brief, the story is one of rapidly increasing prosperity, increased consumption of food in general and fish, pork and chicken in particular, bigger children, the disappearance of nutritional deficiency diseases and—you've guessed it—a rise in the mortality from vascular disease and an increased incidence of gout. Here, indeed, is the full gamut of nutritional science.

The narrative in Yugoslavia is the same but with a difference. Here too a rural population took to the industrial life. The people left the villages to work in the towns where imported food was provided. As they became richer they too ate better while those left behind had a poorer diet. But while this difference persisted and, as Ferber points out, despite the intrusion of combines, cars and television, regional dietary habits and customs remained. Nevertheless, in the country as a whole the consumption of meat, fruit and vegetables rose while the proportion of cereals fell. At the same time pellagra and rickets dwindled away. Ferber surely has the last word when he writes "all efforts of the health policy group would be futile without the advancement of the economic basis of the population".

J. Trémolières, in the third type of article, writes in volume 18, as he has done elsewhere before, to imply that food has a symbolic significance apart from its chemistry and that people are different from the nutritional statistics in which we sometimes purport to describe them. "Reference Man" must always be based on the particular community to which he is intended to be applied.

Finally, there are thirty-five pages in volume 17 by J. E. Gordon and N. S. Scrimshaw—surely written on an off day. This too is a philosophical exposition of their views on how to decide whether action planned to improve a community's nutrition has done any good or not. "Essential components are to establish the goals of professional achievement for nutritional and allied variables, the means for their measurement, a standardisation of performance in their execution and quality control of the assembled data", write the authors or, alternatively, "the approach to evaluation of a nutrition action programme is ecologic, wherein epidemiologic method provides the means to measure effect".

If Dr Geoffrey Bourne, the indefatigable editor of the series, could persuade some of the contributors to knock off a word here and there, he might be able to get the publishers to reduce the price. MAGNUS PYKE

Ways with cells

New Techniques in Biophysics and Cell Biology. Edited by R. H. Pain and B. J. Smith. Vol. 1 pp. 249. (Wiley-Interscience: London and New York, September 1973.) £5.73.

THIS little book clearly emphasises how present-day biology depends on technical innovation. Like all multi-author works, it is heterogeneous in style and approach. Some of the eight chapters describe in detail the theory behind each technique but are thin on useful applications, whereas others list the various problems successfully tackled but with a minimum of theoretical background.

New Techniques begins with an account by J. Edwards of intercellular adhesion in which the Coulter counter is fully exploited to study aggregation of BHK 21 cells, which are the author's speciality. It is followed by a lucid and well-documented description by K. W. Jones of *in situ* DNA-RNA hybridisation, a technique which has already yielded valuable information on gene analysis during development. N. G. Maroudas concludes that there is no "best buy" among the different techniques for anchorage-based cell propagation. There is an authoritative chapter by R. G. Miller on cell separation by velocity sedimentation but the excellent theoretical introduction is not matched by sufficient examples of successful or possible applications of the technique. This is followed by the only two chapters on biophysics—one on circular dichroism as a probe for polysaccharide structure and the other on the application of mass spectrometry to protein sequencing. P. L. Pearson's chapter on differential staining of mammalian chromosomes ends with a useful collection of recipes. The last chapter by S. P. Spragg on on-line computers is more useful for information obtained with physical methods than those of cell biology.

Looking at the book as a whole it is difficult to see the rationale for grouping these five methods of cell biology with two physical techniques, particularly as no mention is made of the contents of future volumes. The index is useful and the presentation of text and figures pleasant but the glossary of fourteen cell biology words seems rather pointless. I feel that this book, limited as it is in its scope, will be useful to many biologists, but hope that the next volume will include techniques such as electron microscopic visualisation of genetic transcription or the application of NMR to biological problems.

J. R. Tata

Organic orbitals

The Organic Chemist's Book of Orbitals. By William L. Jorgensen and Lionel Salem. Pp. xii+305. (Academic (Harcourt Brace Jovanovich): New York and London, May 1973.) $11.50.

There is a growing group of organic quantum chemists who believe that the symmetry properties and shapes of orbitals provide the most informative insight into the structure and reactivity of organic molecules. The concern of the authors of this book is to provide the requisite information in the form of electron density contour diagrams for 104 selected molecules of special interest, from hydrogen to norbornadiene. In each case there are three-dimensional computer-produced drawings for the occupied valence orbitals and for at least one unoccupied orbital, together with their group-theoretical symmetry and the orbital energy. These annotated drawings occupy nearly three-quarters of the book. There is a brief introductory account of molecular orbitals and their genesis from bond orbitals and group orbitals. The remaining pages consist of literature references and indications of the computational methods used (*ab initio* with extended basis sets, MINDO/2 or extended Hückel wave functions). The computer programs for the graphical output devices are not given, nor is there any indication of the effort in terms of programming and computer time.

Any chemist with a weakness for computer graphics should enjoy the pretty drawings produced. While not everyone will share completely the authors' enthusiasm for their usefulness, it is perhaps worth remembering that it can be argued that most progress in theoretical organic chemistry has come through the contemplation of three-dimensional atomic arrangements in molecules. The extension of this approach to electrons is therefore within the tradition of mainstream organic chemistry.

V. Gold

Subatomic interactions

The Pion-Nucleon System. By B. H. Bransden and R. G. Moorhouse. Pp. ix+538. (Princeton University: Princeton, July 1973.) $20 boards; $8.95 paper.

Of all elementary particle interactions, the πN system is undoubtedly the one which has been most extensively studied. This has resulted in a wealth of accurate data for pion-induced reactions, and natural accompaniments have been detailed phenomenological studies, model building, and the testing of theories. This is the field of Bransden and Moorhouse's graduate-level book, although it also contains much material (30–40%) about the techniques of hadron physics in general.

The contents, briefly, are as follows. The book opens with a discussion of basic quantities such as isospin, scattering amplitudes and cross-sections. These are illustrated in Chapter 2 by analyses of low-energy πN scattering and pion photoproduction. Chapter 3 is concerned with fixed-t dispersion relations, starting with a field theoretical derivation of forward dispersion relations for spinless particles, and then discussing applications to πN scattering. Analyticity is developed further in Chapter 4, which contains a review of the analytical properties of amplitudes for potential scattering, and then discusses the relativistic πN case, leading to the Mandelstam representation and partial wave dispersion relations. These four chapters are in general clearly written and straightforward, although the discussion of the Mandelstam representation concentrates exclusively on its analytical properties without a warning statement about the asymptotic behaviour necessary for its existence.

Baryon resonances are the subject of the next two chapters. The first, Chapter 5, contains a detailed account of resonance theory, and explains very clearly the general forms of resonant amplitudes and how they arise. The second half of this chapter describes how resonance parameters are deduced from data by phase shift analysis, and gives an assessment of the results that have been obtained. A somewhat surprising omission is the lack of any description of the actual data themselves, not even qualitatively. The second of these chapters, Chapter 6, discusses the classification of resonances in higher symmetries, starting from the isospin group SU(2) and ending with the L-excitation quark model. There is a good physical discussion of group theoretical ideas, and extensive use is made of Young tableaux. Symmetry ideas are also the subject of Chapter 7 on current algebra and its sum rules. Chapter 8 contains a description of higher-energy two-body scattering, and in particular the ideas of Regge poles and duality, and in Chapter 9 the analyticity approach reappears in a review of some attempts at πN dynamical calculations. The final two chapters, 10 and 11, are concerned with πN inelastic scattering, and a detailed discussion of the form factors of the pion and nucleon, respectively. There are two appendices, on angular momentum and the formalism for pion photoproduction.

In reading this book I was conscious of a certain unevenness in the background required of the reader. For example, some knowledge of field theory is required to understand chapters 3 and 7, whereas isospin is (more than once) introduced without assuming any prior knowledge. This is also true of the style. Undoubtedly the best sections of the book are those where the discussion starts from a comparatively low level and the ideas are developed in detail with the necessary full explanation. Chapters 5, 6 and 11 are of this form, and all are excellent. Less successful are those sections where motivation is lacking, or where discussion terminates at an interesting point. An example of the latter is the discussion of resonance poles on page 185. An example of the former is the concept of "signature". I doubt whether someone meeting the idea, as introduced in section 8.4, for the first time would really appreciate its significance. Graduate students may also be alienated by the fact that words are frequently mentioned long before their meaning is explained; for example, "exotic" and "Veneziano model" (or, occasionally, not explained at all). The readers likely to obtain most from this book are thus those with some prior knowledge of strong interaction physics. They are not likely to be disappointed, for almost all aspects of πN physics are discussed. Certainly, all particle physics libraries should possess a copy.

The volume is well produced, and, since there is a paperback edition, not unreasonably priced. There is a considerable number of misprints, but fortunately most of these are trivial. Despite its typed format, it seems to have taken rather a long time to produce (the preface is dated April 1972) with a result that many of the references have been superseded, but that is probably inevitable in an advancing field like particle physics.

B. R. Martin

Embryos and ethics

The Mammalian Fetus in vitro. Edited by C. R. Austin. Pp. xii+388. (Chapman and Hall: London, July 1973.) £8.

THE *in vitro* culture of mouse and rat embryos during the early somite stages of development has little directly in common with the physiological problems of the premature human infant. It is the aim of *The Mammalian Fetus in vitro* to establish a connection by including both in a series of fields of study, each of which overlaps the next. To further this aim, as the editor explains in his introductory chapter, the meaning of the term 'foetus' has been extended backwards and forwards in time somewhat beyond its usual limits.

Culture of post-implantation stages of rodent embryos through the period of organogenesis is described by D. A. T. New, who himself developed most of the relevant techniques. Later organogenesis is particularly inaccessible in placental mammals, but can be studied in marsupials not *in vitro* but in the pouch. In his chapter on marsupial pouch young, G. B. Sharman presents a comprehensive compilation of data on a topic never previously reviewed. D. A. Nixon deals with the physiology of the mid-term foetus and placenta, mainly on the basis of experiments on foetal lambs. The same theme is considered, with particular reference to the development of an artificial placenta, by W. M. Zapol and T. Kolobow on the foetal lamb and by M. C. Macnaughton on the mid-term human foetus. The problems in this field are enormous, and it is abundantly clear that the vitriparity of Aldous Huxley's *Brave New World* is not yet in sight.

Moving on to the viable (or almost so) stage, C. H. M. Walker and B. J. N. Z. Danesh describe their artificial lung–kidney system as used on puppies and human infants, and E. Hey contributes an outstanding chapter on the problems of the premature baby, including respiration, thermoregulation, circulation and nutrition.

Some of the ethical problems involved are discussed in a final chapter, "The Road Ahead", by R. A. McCance. Clearly the enormous strides that have been made in reducing perinatal mortality would have been impossible without extensive experimentation, on human as well as animal material. Sometimes the measures designed to save the lives of infants introduce new and unsuspected hazards: for example, the hundreds of babies suffering retinal damage and subsequent blindness as a result of excessive oxygen treatment in the early 1950s, or the many deaths from "grey baby syndrome" a few years later, due to injudicious treatment with chloramphenicol.

Guidelines for the use of human foetuses for research have been laid down in the 1972 Report of the Advisory Group set up by the College of Obstetricians and Gynaecologists. Different ethical principles apply to viable and to non-viable infants; in the latter case, as also with animal experimentation, the problem is almost as much aesthetic as ethical. McCance regrets that the Advisory Group recommended that foetuses of more than 20 weeks' gestation should be considered viable, since he feels that this creates a "shadowy period of 8 weeks" during which abortions can be performed, yet the use of the abortuses (almost certainly not viable) for experiments of any kind may become illegal. From the point of view of experiments which could involve pain and distress, the cut-off point should surely come earlier: a 19-week foetus, however non-viable, differs from a 6-day blastocyst in possessing all the mechanisms for feeling pain.

One wider ethical point is not considered in this volume. The survival rate of liveborn babies weighing between 1 and 1.5 kg at birth has been pushed up from 50% to 75%, and 20% even of those weighing less than 1 kg may now "expect to survive the neonatal period with expert care". Fortunately, the outlook for survivors has also improved: past reports on babies weighing less than 1.5 kg at birth suggested that more than half would be handicapped, but more recent reports indicate that "with skilled nursing care less than 20% of the survivors will be seriously handicapped". Even this figure makes a disturbingly high contribution to the total burden of physical and mental handicap. Should lung-kidney machines and all the other achievements of foetal and neonatal physiology be used to try to maintain viability in earlier and yet earlier premature infants? If not, where should the line be drawn?

As one might expect from Professor Austin's editorship, the volume is impeccably turned out.

ANNE MCLAREN

View of the brain

The Understanding of the Brain. By John C. Eccles. Pp. xv+238. (McGraw-Hill: New York and London, May 1973.) £1.95.

THIS short book is based upon a series of lectures delivered at Indiana University early last year. Its aim is to provide an outline of recent neurophysiological work of special relevance to our understanding of brain function and, incidentally, to convey the author's somewhat idiosyncratic views on the relations of brain, mind and consciousness.

Professor Eccles makes it clear that his book is to be regarded not as a text or reference work but as a personal communication limited to aspects of the subject which he regards as of particular interest or importance. These aspects are covered in six longish chapters, dealing respectively with neurones and the nerve impulse, peripheral synaptic transmission, synaptic transmission and pathways in the brain, the control of movement, neurogenesis and the neural basis of learning, and finally, brain, speech and consciousness. A list of selected references, mostly recent though certainly not exhaustive, is appended to each chapter.

The material covered in the earlier chapters is presented with the skill and cogency we have come to expect from Professor Eccles; his account of peripheral transmission, in particular, would seem outstandingly well balanced, particularly when his own changes of view over the years are borne in mind. So, too, is the chapter on the central mechanisms of transmission, in which he goes beyond the experimental data in a praiseworthy attempt to formulate general principles governing central nervous activity. Although it is beyond my competence to assess these chapters at an expert level, they seem to me pertinent, well presented and fair.

The chapter on neurogenesis brings together, in a brief compass, much recent experimental material considered in relation to the pioneer work of such men as Weiss and Sperry. The author defends strongly the view that the developing brain can no longer be viewed as a randomly organised structure moulded by use to an appropriate design but rather as a system in which the basic patterns of connection are established prior to their being called into action. This conclusion obviously has important implications for many problems in genetic neurology.

In his account of movement and its control, Professor Eccles has a great deal more to say about the cerebellum than the motor cortex, though he has little use for those who have attempted to understand its functions on the basis of computer models. For this reason, he makes no reference to the work of Marr and others, which many have found extremely stimulating. It is a pity, perhaps, that this whole approach is dismissed so cavalierly.

The final chapter on mind and brain is inevitably controversial. Rightly, the author devotes considerable attention to the work of Sperry on "split-brain" patients. As is well known, a variety of fascinating disconnections between the activities of the two cerebral hemispheres can be demonstrated in these patients, such that each may under appropriate circumstances operate at a high-order level independently of its fellow. Although the right hemisphere seems to be largely, if not wholly, devoid of linguistic expression, it is nonetheless almost certainly unjustified to treat it, as does Professor Eccles, simply as a "computer" lacking any direct liaison with consciousness. No one who has watched a split-brain patient performing complex visual discrimination and recognition tasks processed wholly by the right hemisphere could possibly doubt the presence of consciousness in the sense in which the term is ordinarily used. It is difficult not to suspect that Professor Eccles's view in the matter is governed, in part at least, by extrascientific considerations.

This book has a glossary though no index. It should make stimulating reading for medical students and others seeking a concise and up-to-date account of several important aspects of contemporary neurophysiology. At the same time, its intentionally selective character will obviously limit its usefulness as a formal text. O. L. Zangwill

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Social wasps and loners

Wasps: An Account of the Biology and Natural History of Solitary and Social Wasps, with Particular Reference to Those of the British Isles. By J. P. Spradbery. Pp. xvi+408 (28 plates). (Sidgwick and Jackson: London, September 1973; University of Washington: Seattle, November 1973.) £8.50; $17.50.

What is a wasp? Dr Spradbery adopts a very narrow definition to include only the members of the Vespoidea, a group which consists of the familiar social wasps and a number of solitary species. In a recent work of very similar title (*The Wasps*, University of Michigan Press, 1970), Evans and Eberhardt take a much wider definition to include all aculeate, or stinging, Hymenoptera, with the exception of the ants and bees. Such a grouping includes the majority of solitary forms (particularly of the Sphecoidea), which have been studied by so many insect ethologists since the early work of J. H. Fabre. Of course, it is quite reasonable to take the more restrictive view, but even Dr Spradbery in his text uses the term frequently in a more general sense.

Taking the Vespoidea then as his group, Dr Spradbery gives us an excellent monographic treatment based mostly on the British species, but with widespread reference to other faunas and to literature otherwise difficult for the non-specialist to obtain. The first part of the book is devoted to a good general discussion of the group, with emphasis on structure and function. There follows a chapter on the solitary species, of the family Eumenidae, concerned mainly with comparative behaviour studies. It is perhaps here that some comparison with the more diverse sphecoid wasps might have been included with advantage. The next eight chapters form the core of the book and are devoted to a thorough review of the behaviour, particularly in terms of nesting, foraging and social organisation, and the population ecology of the British Vespidae, the social wasps.

As would be expected from the author's previous publications, there is a first-class, balanced discussion of many problems, including the controversial field of caste determination. A chapter is devoted to the parasites, predators and commensals associated with wasp colonies. This necessarily is drawn from a wide variety of published records, at least some of which need judging with caution. Chapter twelve is entitled "The Impact of Wasps on Man" and is a useful summary, especially of the sting and its pharmacology. It may come as some surprise to discover that during the period 1949 to 1969, thirty cases were recorded of people in England and Wales who died as a direct result of being stung by wasps! The main part of the book concludes with two chapters of a less detailed nature, which discuss the 800 or so social vespoid species of the world and the possible paths and mechanisms of the evolution of such complex societies.

The book concludes with four very useful appendices. The first is concerned with the taxonomy of the British species and gives good illustrated keys for the identification of the twenty-nine known species. A further appendix gives helpful information on methods of studying, collecting and preserving wasps. The final appendix gives the first published distribution maps for all British species, compiled by the Biological Records Centre, Monks Wood. Clearly these are very incomplete, but their very inadequacy may foster an interest in the group, which is essential for the accumulation of further information.

The standard of line illustrations throughout the book is generally very high. The half-tone plates are in some cases less satisfactory. Most show live wasps or their nests, and others show structural details. A number of good colour plates are included, but those illustrating mounted specimens are rather disappointing. The individual insects are mostly too small to show useful detail and some are very poorly reproduced. Most unfortunately the colour and black-and-white plates are inconsistently laid out, with blank pages interspersed irregularly, for no apparent reason. The legends are often very difficult to associate with the correct figures, and sometimes even hard to find.

In conclusion this is clearly a very valuable and exhaustive work which should be read not only by the small band of research workers interested in wasps, but also by a wider group of general ethologists and ecologists. It will without doubt for long be a standard work in its field.

M. F. Claridge

Straight dislocations

Introduction to Anisotropic Elasticity Theory of Dislocations. By J. W. Steeds. Pp. ix+274. (Clarendon: Oxford; Oxford University: London, May 1973.) £8.35.

In this book Steeds presents a concise, very clearly written account of the elastic theory of straight dislocations in anisotropic media. He concentrates exclusively on those cases whose solution can be analytically expressed, and he shows that these comprise such a large number of cases that the algebraic effort is worthwhile. In short, Steeds 'professionalises' the subject so that a research student with the aid of this book should be able to make intelligent use of anisotropic elasticity, whereas before the book was available most research students (and their supervisors) would founder.

The book contains explicit solutions for a large number of commonly occurring dislocations, and a table at the end lists them so that one can see at a glance whether or not a particular problem is solved. Furthermore, a very clearly written chapter outlines the relationship between the two-dimensional (that is, straight dislocation) solutions and the three-dimensional solutions; it also covers dislocations in uniform motion.

The book is intended to be a reference work, and it has extensive tables of parameters for different crystals. As a reference book, it gains greatly from its clarity and strict deductive approach. As a textbook, it suffers somewhat from these features: students will not easily gain from it a feel for when anisotropic elasticity is important and when it is not; and one would perhaps like guidance on the use of computers to solve some of these problems. In my experience, it is possible to write a computer program which works reliably and which obviates the necessity for algebra or thought—although of course it does not give the kind of insight obtainable from analytic formulae. The computer program itself, however, requires an investment of time which will not pay off unless extensive calculations are envisaged—the one-off calculation is better done by algebra. So there is no doubt that any laboratory concerned with the properties of dislocations should have this book on its library shelves. L. M. Brown

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The commencing salary will be on the scale £1,929 to £2,223 per annum plus F.S.S.U. benefits, and the appointment will be for three years from January 1, 1974 or as soon as possible after that date.

Further particulars and application forms may be obtained from the Registrar/Secretary, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom they should be returned by **Friday, February 1, 1974.**

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Applications are invited for a post of Research Assistant in Earth Sciences, tenable immediately. The person appointed will work under the supervision of Professor I. G. Gass on Petrochemistry and genesis of volcanic rocks. Applicants should possess a good honours degree in Earth Sciences and postgraduate experience/qualifications in geochemistry. The successful candidate will be given the opportunity to register for a Higher Degree of The Open University.

Further details are available from the Acting Personnel Manager (RE 1), The Open University, P.O. Box 75, Walton Hall, Milton Keynes MK7 6AL (Tel. Milton Keynes 74066, extension 3068). Closing date for applications Friday, January 25, 1974. (135)

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Applicants should hold degrees or professional qualifications in Ophthalmic Optics. In the case of the Senior Lectureship, a Higher Degree or Higher Diploma together with appropriate experience will be required.

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Application forms and further particulars may be obtained from The Director, Glasgow College of Technology, North Hanover Place, Glasgow G4 0BA, and should be returned as soon as possible. (149)

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(150)

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(160)

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The salary for Head of School is currently $19,102 per annum (Professorial level).

Further information may be obtained from the Registrar at the address shown below.

Applications containing full personal particulars, details of qualifications and experience, and the names and addresses of two referees, should be forwarded to the Registrar, Queensland Institute of Technology, P.O. Box 246, North Quay, 4000, by February 2, 1974.

(124)

Head of Animal Supplies

The Research Department, situated in Nottingham, has a vacancy for a person to take full charge of the Animal Supplies and Services Unit, with the responsibility for the breeding, purchase, handling and health of a wide variety of laboratory animals for use in medical research. The Unit, comprising small animal, dog and primate sections, is being enlarged. Extensive new animal facilities are planned and the man appointed will be expected to participate in the development of this project. Staff numbers over 25.

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Please write for further details and application form to: J. F. Pattison, Employment Manager,

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(159)

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(103)

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Closing date: **February 28, 1974.**

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Work is expected to range from basic and developmental studies with plant cells in culture, to the transfer and expression of genetic information between cells of related and diverse species (plant-plant and plant-microbial hybrids).

Preference will be given for one position to applicants with a background in plant physiology and genetics and for the second position to applicants with a background in molecular biology of plant and/or microbial DNA and its expression.

Closing date: **February 4, 1974.**

Salaries: Salaries for Professor will be $A20,336 per annum. Salary on appointment to other posts will be in accordance with qualifications and experience within the ranges: Research Fellow $A8,698 to $A11,901 per annum (2 by 641; 2 by 640; 1 by 641); Postdoctoral Fellow $A8,698 to $A11,901 per annum. Current exchange rates are approximately $A1=61 NP=$US1.48.

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Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra. Superannuation (where applicable) is on the F.S.S.U. pattern with supplementary benefits.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF for further particulars before applying. (93)

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A post-doctoral research position for work in association with Professor B. Müller-Hill will be available immediately. The appointment will be for two to three years. The salary will be on the scale of ca. 27,000,— DM per annum.

Applications, with curriculum vitae and the names of two referees, should be sent to Professor B. Müller-Hill, Institut für Genetik der Universität zu Köln, Köln 41, Weyertal 121, West Germany. (25)

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ANIMAL TECHNICIAN (GRADE 3) is required by the Department of Biology for the care of small wild animals, including British reptiles.

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Applicants should preferably have a minimum of one or two years' relevant experience.

The appointment is established with initial salary on the scale £1,650 to £1,920 according to qualifications and experience.

Applications in writing, giving details of age, qualifications and experience and the names and addresses of two referees, should be sent to the Deputy Secretary's Section (Ext. 2400), The University, Southampton SO9 5NH, as soon as possible. Please quote reference number Na/169/T. (92)

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Salary range: £1,929 to £4,548. Duties to commence October 1, 1974. Closing date for applications January 31, 1974.

Applications, together with the names and addresses of two referees, should be forwarded to the Registrar, University College, P.O. Box 78, Cardiff CF1 1XL, from whom further particulars will be available. Please quote reference 0972.

(85)

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The post, tenable for 2½ years, will carry a commencing salary within the scale £2,058 to £2,718 with F.S.S.U. benefits. Further details and application forms from Mr. F. N. Birkett, Department of Mechanical Engineering.

Loughborough Leicestershire

(97)

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Particulars, including information on travel and removal allowances, study leave, housing and superannuation may be obtained from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close on **January 31, 1974.** (174)

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(international publishers of scientific journals and books) has openings in Amsterdam and Oxford for full-time

Editorial Assistants

Description. Preparation of manuscripts for printing, correction of proofs, language correction of contributions written by non-English speaking authors, supervision of freelance workers, responsible and varied work.

Requirements. Degree or equivalent in chemistry, biochemistry, or biomedicine preferred, English mother tongue, meticulous eye for detail.

A good salary according to qualifications and experience, work in a congenial atmosphere, and excellent social security arrangements are offered.

Handwritten applications giving details of education and experience and an indication of salary expected should be addressed to The Personnel Department, Associated Scientific Publishers, P.O. Box 2400, Amsterdam, The Netherlands.

(122)

TROPICAL PRODUCTS INSTITUTE

Animal Nutritionist

. . . to lead small group engaged in assisting developing countries on the better utilisation of animal feed resources and the development of feed manufacturing industries.

The post, in London, will entail travel overseas both to advise governments of developing countries and to undertake project work on animal feed development.

Candidates (aged at least 25 and normally under 32) should have a 1st or 2nd class honours degree, or equivalent, in a relevant subject, and have at least four years' postgraduate experience. Practical experience in the analysis of feed materials an advantage, and experience of applied animal nutrition essential.

Appointment will be as Senior Scientific Officer (£2,790–£3,815).

Application forms (to be returned by 1 February 1974) from Miss C. A. Hall, Administrative Secretariat, Tropical Products Institute, 127 Clerkenwell Road, London EC1; please quote SA/10/JD.

(82)

LIVERPOOL REGIONAL CARDIAC CENTRE

SEFTON GENERAL HOSPITAL, Liverpool L15 2HE

Applications are invited for the post of

BIOCHEMIST

(Basic Grade, Probationary or Post Probationary) in the Research Laboratory of the Liverpool Regional Cardiac Centre. The work of the laboratory covers research into all aspects of heart disease with particular emphasis on coronary artery disease.

Opportunities exist for studying for a higher qualification. The appointment will be subject to the Whitley Council terms and conditions of service.

Enquiries should be made initially to Dr T. Deegan, at the above address (Tel. 051-733 4020 ext. 311). Application form obtainable from the Acting Secretary, The United Liverpool Hospitals, 80 Rodney Street, Liverpool L1 9AP, to be returned by January 25, 1974. (79)

UNIVERSITY OF EDINBURGH
FACULTY OF MEDICINE

Chair of Rehabilitation Studies

The University Court invites applications for the Chair of Rehabilitation Studies which has been established by means of gifts from the National Fund for Research into Crippling Diseases and the Thistle Foundation. The Chair has been founded primarily to foster research in the many aspects of rehabilitation. It is confidently expected, however, that the involvement of the Professor and his colleagues in undergraduate and postgraduate education will lead to a greater realisation within the health professions and the community of the importance of rehabilitation and resettlement after all forms of illness and disability.

The Professor is initially to be accommodated within the Department of Orthopaedic Surgery but is in no sense restricted to the problems of locomotor rehabilitation.

The appointment will be made in association with the South-Eastern Regional Hospital (Scotland) Board (or its successors from 1st April, 1974) who have undertaken to fund two consultant posts in rehabilitation medicine and supporting staff.

Applications will be welcomed from candidates in any discipline within the health professions. It is hoped that the Professor will be able to take up office by October, 1974.

The stipend of the Professor will be £7,599 per annum, and membership of F.S.S.U. will be required.

Further particulars and terms of appointment may be obtained from the undersigned to whom applications (16 copies), giving the names of three persons to whom reference may be made, should be lodged not later than 16th March, 1974.

Secretary to the University,
University of Edinburgh,
Old College,
South Bridge,
Edinburgh EH8 9YL

(126)

GLASSHOUSE CROPS RESEARCH INSTITUTE

Applications are invited for the following posts:

1. **Two SCIENTIFIC OFFICER/HIGHER SCIENTIFIC OFFICERS in PLANT BREEDING DEPARTMENT** for work on vegetable crops. Good honours degree required in a biological subject with specialisation in genetics, coupled with plant pathology or plant physiology. These posts are being re-advertised. Closing date February 14.

2. **One HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER in BIOCHEMISTRY AND CROP PROTECTION DEPARTMENT** to lead team working on growth regulating substances. Applicants should have good honours degree or equivalent qualification in plant chemistry or other appropriate subject with at least two years' post-graduate research or development experience. At least four years relevant post-graduate research or development experience required for appointment to SSO grade. Closing date February 21.

Salary scales: £1,590–£2,177 (SO); £2,076–£2,667 (HSO); £2,615–£3,640 (SSO); Additional non-pensionable allowance of 4¼% of gross salary to offset superannuation contributions.

Further details obtainable from Secretary of the Institute, Worthing Road, Rustington, Littlehampton, Sussex, to whom applications should be sent, giving full biographical details and the names and addresses of two referees.

(165)

Horticultural Inspector

Scotland £3377-£4057

for work in connection with schemes relating to improvement, business grants, fruit certification and bulb inspection and to carry out import and export plant inspection, horticultural surveys and liaison with various horticultural organisations and outside interests.

Candidates (aged at least 26) should, preferably, have a degree or national diploma in Horticulture together with practical knowledge and experience of commercial horticulture. Specialist knowledge of fruit and bulb production and a working knowledge of Government grant-aided schemes would be an advantage. Ability to drive a car essential.

Starting salary £3,377 on a scale rising to £4,057. Prospects of promotion. Non-contributory pension scheme.

For full details and an application form (to be returned by February 1, 1973), write to Scottish Office, Personnel Division, St. Andrew's House, Edinburgh EH1 3BX quoting S/8499.

Department of Agriculture and Fisheries for Scotland

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UNIVERSITY OF THE ORANGE FREE STATE

C.S.I.R. FLAVONOID RESEARCH UNIT

RESEARCH ASSISTANTSHIP

Applications are invited for the above vacancy in the Department of Organic Chemistry tenable over a period of up to 3 years. The work involves postgraduate research in all branches of flavonoid and tannin chemistry leading to M.Sc. or Ph.D. degrees, according to initial qualification. Preference will be given to candidates for the Ph.D. degree. The position should be assumed as soon as possible after January 1, 1974.

Minimum initial salary with M.Sc. qualification: R3,000; B.Sc.(Hons.) R2,500.

Applications, giving details of academic record and curriculum vitae should be posted to Professor D. G. Roux, Department of Chemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, South Africa. (31)

UNIVERSITY OF ZAMBIA

Applications are invited for (a) SENIOR LECTURESHIP or (b) LECTURESHIP IN PHARMACOLOGY in the School of Medicine. Duties will include the teaching of physiological pharmacology to pre-clinical students, organisation of the practical work, teaching of chemotherapy to clinical students and the carrying out of pharmacological research of relevance to the continent. Appointee will be required to take up duties as soon as possible. Salary scales: Senior Lecturer K5,600 to K6,600 per annum. Lecturer K4.000 to K5,400 per annum. There is a medical supplementation of K1,200 per annum (£1 sterling=K1.50.) The British Government may supplement salaries in range £200 to £500 per annum (sterling) for married appointees, or £100 to £200 per annum (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages; various allowances; superannuation and medical aid schemes; regular overseas leave.

Detailed applications (two copies), including a curriculum vitae and naming three referees, should be forwarded by airmail, not later than January 26, 1974, to the Registrar, University of Zambia, P.O. Box 2379, Lusaka, Zambia. Applicants resident in U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (173)

UNIVERSITY COLLEGE, DUBLIN

DEPARTMENT OF GEOLOGY

COLLEGE LECTURER
or
ASSISTANT LECTURER

Applications are invited for the above post by geologists with specialist qualifications in Sedimentary Petrology and Stratigraphy.

The essential requirements are a Doctorate, together with adequate experience and research accomplishments, and the names of two referees with personal knowledge of the applicants must be submitted.

Salary scale: College Lecturer: £3,232 by £154 to £4,310; Assistant Lecturer: £2,195 by £122 to £3,049. Non-contributory pension and family allowances are additional to salary.

Applications should be made before February 1, 1974, to J. P. MacHale, Secretary and Bursar, University College, Belfield, Dublin, from whom further details may be obtained. (156)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF MEDICINE
(LEEDS GENERAL INFIRMARY)

Applications are invited for the post of RESEARCH ASSISTANT in MYCOLOGY in the dermatology section of the department. The successful candidate will take part in studies on the epidemiology and pathogenesis of Candida infections. Applicants should have a degree in Microbiology or a related subject. The appointment will be made for one year in the first instance, and it is hoped that the successful candidate will take up the appointment as soon as possible. Starting salary £1,449 (with F.S.S.U. benefits).

Forms of application and further particulars from the Registrar, The University, Leeds LS2 9JT (please quote reference 50/5/D). Closing date January 31, 1974. (172)

Research Unit on the Spatial Structure of Biopolymers

A research unit on the spatial structure of ribosomes and their components is to be established at the Max-Planck-Institut für Molekulare Genetik in Berlin-Dahlem, with the financial support of the Deutsche Forschungsgemeinschaft.

Applications are invited for the positions of the head of this research unit and for scientists working in the unit. The annual salary for the head will be approximately 45-50,000 DM and for the scientists 32-42,000 DM. The initial appointment will be for three years with the possibility of extension.

Applicants should have experience in one of the following fields: Crystallization of proteins or nucleic acids, X-ray crystallography of biopolymers, chemistry of proteins or nucleic acids. It is not necessary for the applicants to be German.

Applications should be sent before March 30, 1974, to Professor H. G. Wittmann, Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 63-73, D-1000 Berlin 33, Germany.

(112)

UNIVERSITY OF OTAGO

FACULTY OF MEDICINE

THE CHRISTCHURCH CLINICAL SCHOOL

CHRISTCHURCH, NEW ZEALAND

RESEARCH OFFICER/ SENIOR RESEARCH OFFICER

Applications are invited from suitably qualified persons for the position of Research Officer in the Department of Surgery within the Faculty of Medicine.

Applicants should hold a Ph.D. or its equivalent and postdoctoral research experience is desirable. Applicants should have a sound scientific background and preferably some experience within the broad field of transplantation and tumour immunobiology.

Salary scale: Research Officer NZ$5,985 to NZ$8,343 per annum.

A particularly well-qualified candidate could be appointed at the level of Senior Research Officer on a salary scale ranging from NZ$8,489 to NZ$10,846 per annum, with a bar at NZ$9,963 per annum. Salary scales are subject to both triennial review and cost-of-living adjustments.

Further particulars are available from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University, P.O. Box 56, Dunedin, New Zealand. Particular enquiries concerning the position may be addressed to Professor W. A. A. G. Macbeth, Department of Surgery, Christchurch Clinical School, Christchurch Hospital, Private Bag, Christchurch, New Zealand.

Applications close in London and in New Zealand in **January 31, 1974.** (98)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF GENETICS

Applications are invited for the post of LECTURER IN POPULATION GENETICS in the Department of Genetics.

Initial salary within the range £1,929 to £4,548 per annum, according to qualifications and experience.

The post is tenable from October 1, 1974.

Applications, together with the names of three referees, should be received not later than February 27, 1974, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref. RV/N/80640. (129)

UNIVERSITY OF WARWICK

RESEARCH TECHNICIAN GRADE 3

The Department of Biological Sciences has a vacancy for a permanent post in the research laboratories. The person appointed will work on the biochemistry of proteins and nucleic acids, under the direct supervision of a member of the academic staff.

Applications in writing to the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No. 19/2T/73, as soon as possible. (87)

GOVERNMENT OF FIJI

HYDROLOGIST

Required for the Public Works Department for one tour of 30/36 months' initially. Salary including TAX FREE SUPPLEMENT will be in the range £2,180–£3,180 a year according to qualifications and experience. Other benefits include a substantial gratuity, free passages, liberal leave on full salary, generous education allowances and Government quarters at moderate rental. An appointment grant £200, car advance £600 and packing expenses £60 may be payable.

Candidates MUST have a wide experience ranging over 10 years in general hydrological works, with particular emphasis on the collection, collation and evaluation of rainfall and run-off data, and the location and establishment of hydrological stations.

Because of lower rates of income tax in Fiji, the gross emoluments are roughly equivalent to UK salaries of

£3,200 to £4,600 for a single man

£3,400 to £4,800 for a married man with 2 children

The post described is partly financed by Britain's programme of aid to the developing countries administered by the Overseas Development Administration of the Foreign and Commonwealth Office.

For further particulars you should apply, giving brief details of experience to:

crown agents

M Division, 4 Millbank, London SW1P 3JD, quoting reference number M3F/730217/NF.

(147)

MINISTRY OF AGRICULTURE FISHERIES AND FOOD LONDON

CHIEF SCIENTIST'S GROUP

ASSISTANT RESEARCH LIAISON OFFICERS

There are vacancies at SCIENTIFIC OFFICER level in the Chief Scientist's Group which is responsible for providing scientific advice to the Ministry on policy matters relating to agriculture, horticulture and food science, for framing, commissioning and reviewing the Ministry's research and development programme and for keeping in touch with R and D work in the UK and overseas.

The disciplines involved are Animal Husbandry, Arable Crops and Plant Science, Horticulture, Agricultural Engineering, and Food Science and Technology.

The appointment will be for a period of three years. Duties include general scientific support to the Research Liaison Officers (Senior Principal Scientific Officers): drafting papers for R and D Boards and Committees of the Joint Consultative Organisation: preparation and evaluation of statistical information and interpretation of reports, data, scientific papers and computer printouts: visits to Research establishments, NO laboratory work.

QUALIFICATIONS: University Degree, HNC or equivalent qualification in an appropriate scientific subject.

AGE: Normally under 27 years but older candidates will be considered.

SALARY SCALE: £1,318 to £1,402 to £1,499 to £1,590 by nine increments to £2,177 plus £175 per annum. Inner London Weighting. Starting salary determined on basis of qualifications and experience.

APPLICATIONS: Fuller details of the posts and application forms are available from Mrs. M. Lyn-Cook, Ministry of Agriculture, Fisheries and Food, Room 625, Great Westminster House, Horseferry Road, London SW1P 2AE. Telephone No. 01-834 8511 Extn. 6449 or 6244. Closing date for completed applications January 23, 1974.

(142)

Warrnambool Institute of Advanced Education

Post Office Box 423,
Warrnambool 3280
Victoria, Australia

The Institute is a Regional College of Advanced Education providing diploma courses in applied science, art, business studies, engineering and social science. A degree course in business will be introduced in 1974. A degree course in social science is at an advanced planning stage. Other degree courses and courses in teacher education will be introduced as soon as possible.

Plans are now being prepared for complete relocation on a 150-acre site. The first residence buildings on the new site were occupied in February, 1973.

HEAD. OF SCHOOL/DIVISION

(Two Positions)

1. Division of Applied Science and Engineering.
2. Division of Business Studies, General Studies (including Social Science) and Art and Design.

SALARY: $19,102 per annum.

DUTIES: Each head will be responsible to the principal for the administration of his division. He will be expected to provide academic leadership and enrichment by teaching, investigation and publication in his discipline. He will be expected to work in collaboration with heads of faculty and other staff for the maintenance and advancement of the academic programme of the Institute.

QUALIFICATIONS: Recognised scholarship together with a higher degree or equivalent in or related to one or more of the disciplines of the division.

At least 15 years' relevant professional experience, including some experience in tertiary education.

Ability and experience in the development and administration of academic programmes.

APPLICATION: Prospective applicants should obtain further particulars from the Academic Registrar.

Applicants will be expected to include particulars of qualifications, experience and present position, together with the names of three referees.

Applications close with the Academic Registrar on Monday, February 18, 1974.

(123)

UNIVERSITY OF ABERDEEN

RESEARCH ASSISTANTSHIP IN CHEMISTRY

Applications are invited for above post for work on organic reactive intermediates in collaboration with Dr A. R. Forrester. Salary £1,896 per annum; appointment for one year only.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) giving the names of two referees should be lodged as soon as possible. (125)

MARINE SCIENCE TEAM

Geological Oceanographer-Geophysicist, Ph.D., 20 years' experience in continental shelf exploration, research and teaching, with numerous publications and some patents pending, and **Biological Oceanographer,** M.Sc., specialist in marine microbiology and pollution, presently teaching and operating own research vessel in the eastern Mediterranean, are interested in becoming associated with an aggressive, developing research, academic or industrial organisation, preferably Europe or Mediterranean area.—Write Box 2079, Nature, T. G. Scott & Son Ltd., Clement's Inn, London WC2A 2ED. (7)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)

Applications are invited for the post of

LECTURER IN VIROLOGY

(in the Department of Medical Microbiology)

Preference will be given to those with medical degrees.

Salary scale: (a) £3,012 to £4,668 (Clinical); (b) £1,929 to £4,548 (Non-clinical) plus £162 London allowance.

The School provides a routine hospital diagnostic service and the successful candidate will be expected to participate in this work as well as undertaking research and teaching. The hospital has its own infectious diseases unit and there are excellent opportunities for gaining wide experience in clinical virology, as well as in diagnostic bacteriology both in the Department and in the associated Public Health Laboratories.

Applications, including curriculum vitae and names and addresses of two referees, to the Dean, St. George's Hospital Medical School, Blackshaw Road, Tooting, London SW17 0QT, before February 8.

The Virology Unit is at Hyde Park Corner and may be visited by arrangement with Professor H. Stern. (119)

MONASH UNIVERSITY

MELBOURNE, AUSTRALIA

DEPARTMENT OF PHYSICS

LECTURER

Applications are invited from experimental physicists with a research interest in one of the following areas: low temperature physics (helium studies, nuclear orientation and superconductivity), laser studies, spectroscopy (Mössbauer, ESR, NMR, optical), fundamental magnetic, electrical and structural studies of matter including polymers, minerals and biological materials.

The Department which has 72 staff members, is well equipped for research in these areas and has good technical services including mechanical and electronics work-shops, materials preparation, computing and photographic facilities.

Salary scale: $A8,698 to $A11,982 per annum with superannuation based on an endowment assurance scheme, the employee and employer contributing 5 per cent and 10 per cent respectively.

Benefits: Travelling expenses for appointee and family; removal allowance; repatriation after three years' appointment if desired; temporary housing for an initial period. Study leave entitlement accumulates at the rate of one month's leave for each six months' service up to six years, with provision for financial assistance.

Further general information and details of application procedures are available from the Academic Registrar, Monash University, Wellington Road, Clayton, Victoria 3168, Australia, or the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF. Technical enquiries should be addressed to Professor R. Street, in the University.

Closing date: **February 22, 1974.**

The University reserves the right to make no appointment or to appoint by invitation. (101)

ROYAL POSTGRADUATE MEDICAL SCHOOL

SCIENTIFIC OFFICER (Graduate in Biochemistry, Chemistry or Biological Sciences) required to assist research biochemist investigating prostatic tissue. Training given in advanced laboratory techniques The research project involved may yield material suitable for submission for a higher degree. Salary not less than £1,812 per annum.

Applications to the Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS, quoting ref. no. 3/326/N. (141)

PERSONAL ASSISTANT/ SECRETARY

Graduate, required by the Director of Research and Managing Director. Shorthand or audio-typing essential. An interest and some basic training in Scientific/Medical subjects essential. Company engaged in Drug Research. Very interesting work and excellent conditions, with pension and assurance scheme in operation.

Apply BIOREX Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB. (167)

The University of Leeds
DEPARTMENT OF PHYSICS

Applications are invited for the vacant CHAIR of Theoretical Physics in the Department of Physics. The salary will be not less than £5,625 per annum and the appointment will be made from a date to be arranged with the successful candidate. The University reserves the right to consider for appointment persons other than those who submit formal applications.

Applications (two copies) stating age, qualifications and experience naming three referees, should reach the Registrar, The University, Leeds LS2 9JT (from whom further particulars may be obtained), quoting the reference number 22/13/D, not later than March 4, 1974. Applicants from overseas may apply in the first instance by cable naming three referees who should preferably be in the United Kingdom. (120)

UNIVERSITY OF BOTSWANA, LESOTHO AND SWAZILAND

Applications are invited for the following post:

CHAIR OF MATHEMATICS. The University is seeking a versatile mathematician with interests in undergraduate teaching and research relevant to the needs of the three developing countries it serves. A particular interest in the development of courses appropriate to middle-level manpower training would be welcomed. Two years of the University's four-year post-O-level programme are at present taught in all three countries. The full programme in Mathematics is at present offered only in Lesotho and the Chair is likely to be tenable on the Lesotho campus.

The appointment will be on contract for limited periods, normally for three years in the first instance. Appointee will be expected to assume duty by July 1, 1974, or as soon as possible thereafter. Salary scale: R5,880 to R6,420 per annum (£1 sterling=R1.55). A 25 per cent gratuity is paid in lieu of superannuation. The British Government may supplement salary by £1,000 per annum (sterling) for married appointee or £600 per annum (sterling) for single appointee (normally free of all tax) and provide children's holiday visit passages. Education allowance; medical scheme; accommodation at reasonable rents; family passages; biennial overseas leave.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees should be forwarded by air mail, not later than January 30, 1974, to the Registrar, University of Botswana, Lesotho and Swaziland, Roma, Lesotho, Africa. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars of the appointment may be obtained from either address. (137)

SUNDERLAND POLYTECHNIC
DEPARTMENT OF MINING

Applications are invited for the post of

CURATOR

N.J.C., A.P.T. & C. Staff T4 (£1,644 to £1,926) in Geology in the Mining Department. Candidates should either be graduates or have wide experience in Geology.

Applications are restricted to Local Government Officers in England and Wales (excluding London).

An application form and further particulars can be obtained from the Personnel Officer, Sunderland Polytechnic, Chester Road, Sunderland SR1 3SD. The form should be returned by January 25, 1974. (102)

COMMONWEALTH AGRICULTURAL BUREAUX

Vacancy for

SCIENTIFIC INFORMATION OFFICER

at the

COMMONWEALTH BUREAU OF PLANT BREEDING AND GENETICS
DEPARTMENT OF APPLIED BIOLOGY
DOWNING STREET, CAMBRIDGE

Duties: Duties include preparation and editing of abstracts for the journal *Plant Breeding Abstracts*, which covers world literature on plant breeding and genetics.

Qualifications: Applicants should have a degree in botany, agriculture or genetics. A knowledge of or aptitude for foreign languages would serve as an additional qualification. An ability to write good English is essential.

Salary: In the scale £1,318 to £2,422 (bar) to £2,615 to £3,250 (bar) to £3,510 to £3,742, plus a compensatory allowance (taxable but not superannuable) of 4½ per cent to offset personal contribution to F.S.S.U. Starting salary according to qualifications, experience and age.

Application forms and full particulars from the Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN.

Closing date for applications: January 31, 1974. (104)

AUSTRALIA
Public Service of Victoria
Ministry for Conservation
Fisheries and Wildlife Division

Research Officer

Ref. No. (P/01)

TWO POSITIONS

Yearly Salary: $A6,229 minimum; $A10,674 maximum. (Generally appointment will be within the salary range $A6,229 to $A8,981 according to qualifications and experience, but applicants possessing special qualifications or extensive experience may be considered for appointment to the range $A9,151 to $A10,674).

POSITION NO. 1

Duties: To carry out field and laboratory studies in marine primary productivity and energetics in relation to water quality characteristics; other research duties as directed.

Qualifications: An approved degree in Science with appropriate training in Botany; appropriate training and experience as related to primary productivity and energetics is desirable. Experience with productivity of higher plants an advantage.

POSITION NO. 2

Marine Invertebrate Zoologist, Western Port Bay Environmental Study

Dutes: As a Marine Invertebrate Zoologist to conduct investigations into the quantitative ecology of Westernport Bay with a view to assessing the effects of industrial and domestic development in the area; other research duties as directed.

Qualifications: An approved degree in Science with Zoology as a major; appropriate training and experience in the taxonomy of polychaetes.

Note: Separate applications must be submitted for these positions.

Applications should be addressed to the Secretary, Public Service Board of Victoria, State Public Offices, 1 Treasury Place, Melbourne, 3002, Australia, quoting reference number (P/01), not later than Tuesday, January 29, 1974, together with statements of experience and qualifications and date and place of birth. (105)

UNIVERSITY COLLEGE, DUBLIN
GEOLOGY DEPARTMENT
RESEARCH ASSISTANTS
1973–4 SESSION

Applications are invited for a Research Assistantship in the current session, with the possibility of re-appointment in the two succeeding sessions. The Assistants will be required to carry out research on the Igneous and Metamorphic Geology of the Leinster Massif, under the sponsorship of the National Science Council of Ireland.

The essential requirement is a good Honours degree in Geology and preference will be given to candidates with ability to conduct research, and with experience in XRF work. Successful applicants who do not hold doctorate degrees will be expected to register for a Ph.D. of the National University of Ireland.

Salary: approximately £900 per annum.

Applications must be submitted before January 31, 1974, but appointments may be made before that date.

Apply to: the Secretary, Department of Geology, University College, Stillorgan Road, Belfield, Dublin 4. (157)

UNIVERSITY OF OTAGO
DUNEDIN, NEW ZEALAND
LECTURER OR SENIOR LECTURER IN MICROBIOLOGY

The Council of the University of Otago, Dunedin, New Zealand, invites applications from Science graduates for an appointment as Lecturer or Senior Lecturer in the Department of Microbiology in the Medical School.

Salary: According to qualifications and experience: Lecturer: NZ$6,575 to NZ$8,343 per annum; Senior Lecturer: NZ$8,489 to NZ$10,846 per annum with a bar at NZ$9,963 per annum.

Further information may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, from Professor J. A. R. Miles, Department of Microbiology, University of Otago Medical School, P.O. Box 913, Dunedin, New Zealand, or from the Registrar of the University, P.O. Box 56, Dunedin, New Zealand.

Applications close in London and in New Zealand on **February 9, 1974.** (99)

OPPORTUNITIES IN FORENSIC SCIENCE

Chemists and Biologists graduating this year or completing a post-graduate course

Science has important contributions to make to the administration of justice and the investigation of crime. The Forensic Science Service is expanding to meet increasing police demands and to take advantage of current scientific developments.

Vacancies are expected in the regional laboratories, where the young scientist will at first assist experienced colleagues and later assume responsibility for examining material associated with crime, preparing reports and giving evidence in court. Posts should also become available in the Central Research Establishment at Aldermaston which provides R & D facilities, central analytical services and computer based information retrieval.

Appointment will be as Higher Scientific Officer (£2076-£2667) or Scientific Officer (£1318-£2177), depending on age, qualifications and experience. Starting salary may be above the minimum of the scale at each level.

A degree/HNC/HND in an appropriate subject is essential. For HSO at least 2 years postgraduate experience is necessary, and candidates should normally be aged under 30. For SO they must be under 27.

For further details and an application form (to be returned by 1st February 1974) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB or telephone Basingstoke 29222 Ext 500 or London 01-839 1992 (24 hour answering service). Please quote: SB/7/LA.

Home Office

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UNIVERSITY OF KENT AT CANTERBURY

RESEARCH ASSISTANT IN THE BIOLOGICAL LABORATORY

A RESEARCH ASSISTANT is required for metabolic studies of biodegradation of detergent compounds by bacteria. Applicants should have either a degree in microbiology, or alternatively, in biochemistry with some experience in the handling of micro-organisms. Salary range £1,311 to £1,497.

Applications with full details of qualifications and experience and with the names of two referees to the Assistant Registrar, Faculty of Natural Sciences, The University, Canterbury, Kent, by January 16, 1974, quoting Ref. A45/73. (109)

UNIVERSITY OF MANCHESTER

RESEARCH ASSISTANT IN THE DEPARTMENT OF CHILD HEALTH

Applications invited for this post from graduates, preferably with post-doctoral experience, to work on tissue culture of endocrine pancreas. Experience in cell biology, endocrinology or biochemistry an advantage. Appointment is for two years from March 1, 1974. Salary range £2,718 to £3,048 per annum. F.S.S.U.

Particulars and application forms (returnable by January 18, 1974) from Dr. R. D. G. Milner, St. Mary's Hospital, Manchester M13 0JH. (60)

UNIVERSITY OF EXETER

DEPARTMENT OF MATHEMATICS

N.E.R.C. RESEARCH ASSISTANTSHIP

Applications are invited from suitably qualified candidates for the post of Research Assistant in the Department of Mathematics. The post is tenable initially for a two-year period (approximately) from the date of appointment; the work is concerned with numerical mathematical modelling of large-scale flow in the atmosphere, and is a research project supported by N.E.R.C. under the direction of Professor D. R. Davies. Candidates should be graduates with qualifications in Mathematics or in Meteorology.

Salary scale: £1,398-£1,482-£1,578 with F.S.S.U. benefits.

Further particulars may be obtained from the Secretary of the University, Northcote House, The Queen's Drive, Exeter EX4 4QJ, to whom applications giving the names of two referees, should be forwarded (4 copies; overseas candidates 1 copy) by February 23. Please quote Ref.: 1/62/7063. (88)

IMMUNOLOGICAL TECHNICIAN

required to work on various aspects of cell mediated immunity. Salary on Whitley Council scale for Medical Laboratory Technicians, i.e., £1,440-£2,292 per annum, plus £126 L.W.

Applications and enquiries to: Dr H. E. Amos, Department of Immunology, Institute of Dermatology, St. John's Hospital for Diseases of the Skin, Homerton Grove, London E9 6BX. Tel.: 01-985 7061. (89)

CROSS INFECTION REFERENCE LABORATORY

We have a vacancy for a Technician or Senior Technician in the Streptococcus Laboratory. This post would be suitable for a candidate who is working for the I.M.L.T. Special Examination. Graduates may apply for this position. Salary according to qualifications and experience, National Health Service terms and conditions of service and superannuation.

Applications to Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, stating age, qualifications and experience and names and addresses of two referees. (134)

UNIVERSITY OF OXFORD

Applications are invited for a post of UNIVERSITY LECTURER IN THEORETICAL PHYSICS from October 1, 1974, at a salary on the scale £1,929 per annum (at the age of 24 or under) to £4,863 per annum (at the age of 42 or over).

The lecturer will be expected to participate in the teaching and research work of the Department of Theoretical Physics.

The successful candidate may be elected, if a man, to a Fellowship at St. John's College, or if a woman, to a Fellowship at Somerville College. Details of the college duties and stipends may be obtained from the Senior Tutor of St. John's College or the Principal of Somerville College, but no separate application for a College Fellowship need be made.

Further particulars may be obtained from Professor Sir Rudolf Peierls, Department of Theoretical Physics, 12 Parks Road, Oxford OX1 3PQ, to whom applications (5 copies, except from overseas applicants) should be sent by February 2, 1974. (95)

UNIVERSITY OF OXFORD

DEMONSTRATORSHIP IN DEVELOPMENTAL BIOLOGY

The Lincare Professor of Zoology proposes to appoint, on or before September 1, 1974, a Departmental Demonstrator in Developmental Biology. The salary will be on a scale rising to £2,283 (with F.S.J.M.).

It is hoped that the Demonstrator will cooperate with Dr R. L. Gardner and Dr C. F. Graham in the teaching of Reproduction and Developmental Biology. Research facilities are available for the Demonstrator.

Applications (if possible, seven copies, except in the case of overseas applicants who need only submit one copy) should be sent to Professor J. W. S. Pringle, Department of Zoology, South Parks Road, Oxford OX1 3PS, not later than February 28. They should include a statement of age, qualifications and experience, and the names of two referees. (106)

PAHLAVI UNIVERSITY

COLLEGE OF ARTS AND SCIENCES
SHIRAZ, IRAN

Applications are invited for positions in Astronomy and Physics beginning fall 1974, at the ASSISTANT/ASSOCIATE PROFESSOR level. Duties include teaching B.Sc. and M.Sc. students and some research. Field of solid state, nuclear, and astrophysics are represented in the department. A Varian X-band E.P.R. Spectrometer is in operation, and a 20-inch Cassegrain reflector with a photoelectric photometer is expected to be in operation by July 1974.

Ph.D. is required. Appointments will be made on a two-year renewable contract basis with an annual salary range equivalent to $12,000 to $16,000 depending on qualifications and experience. Return fare included.

Send résumé, curriculum vitae and confidential recommendations from three references to Dean Vessal, from whom particulars may be obtained. Deadline for applications is April 1, 1974. (107)

ST. BARTHOLOMEW'S HOSPITAL

LONDON, E.C.1

GASTROENTEROLOGY ASSISTANT required to help and supervise patients for investigations. Medical qualifications not necessary.

Salary scale £708 to £1,335 per annum plus £126 London Weighting.

Please apply in writing to the Personnel Officer, quoting ASC/3872/N. (108)

WESTERN AUSTRALIA KINGS PARK BOARD

invites applications for the position of RESEARCH OFFICER. Applicants should hold preferably a higher degree in Botany, Horticulture or a related subject. The appointee will be required to participate in a programme of research under the supervision of the Director on the nutrition and environmental requirements, germination, growth, reproduction and dispersal mechanisms of native plants.

The appointment is subject to the conditions of the State Public Service and the present salary range is $A6,767 to $A9,263 per annum.

Further information concerning King's Park and Botanic Garden or the position of Research Officer may be obtained from the Board. Applications, including details of the applicant's career, qualifications and the names and addresses of two referees (please do not send original certificates or testimonials) should reach the Director not later than January 31, 1974, Kings Park Board, West Perth, Western Australia 6005. (148)

HOUGHTON POULTRY RESEARCH STATION

Applications are invited for the post of

SENIOR VETERINARY RESEARCH OFFICER
OR
PRINCIPAL VETERINARY RESEARCH OFFICER

Applications are invited for a virologist with veterinary qualifications to be responsible for the study of avian respiratory viruses. The appointment will be made within either the Senior Veterinary Research Officer scale (£3,095 by 7 to £3,742) or the Principal Veterinary Research Officer scale (£3,963 by 6 to £5,202) depending upon qualifications and experience.

F.S.S.U. superannuation, membership of which attracts a non-pensionable allowance of 4½ per cent of salary.

Further particulars and application forms available from the Secretary, Houghton Poultry Research Station, Houghton, Huntingdon PE17 2DA. Closing date for applications February 11, 1974. (14)

UNIVERSITY OF EDINBURGH

TECHNICAL OFFICER ELECTRON MICROSCOPY

Required for the Department of Botany; to be responsible for the operation of the microscope and for assisting and advising research staff on electron microscope techniques. Applicants should be suitably qualified and familiar with the preparation of material for electron microscopy, particularly in ultramicrotomy. Salary range £2,007 to £2,382 per annum. Ref. no. T5305.

Written applications, quoting the post reference number, should be addressed to the Personnel Officer, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS. Telephone number 031-667 1011, ext. 4325. (116)

THE UNIVERSITY OF ADELAIDE

DEPARTMENT OF PHYSICAL AND INORGANIC CHEMISTRY

TEACHING FELLOWS

Applications are invited for Teaching Fellows who should have a Ph.D. degree in either Physical or Inorganic Chemistry. Half of his time will be devoted to teaching and half to research. A document detailing research interests of the Department will be sent on application.

Salary scale: $A7,270 by $A286 (3) by $A285 (2) to $A8,698. Initial salary will be fixed in relation to qualifications and previous experience. No provision is made for superannuation.

Further information, including list of particulars required of an applicant, is available from the Registrar of the University or from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications should reach the Registrar, G.P.O. Box 498, Adelaide, S.A. 5001, not later than **February 1, 1974.** (136)

NATIONAL INSTITUTE FOR BIOLOGICAL STANDARDS AND CONTROL

HAMPSTEAD, LONDON

JUNIOR TECHNICAL OFFICER/TECHNICIAN required in the Antibiotics Division to work in a laboratory concerned with the evaluation and control of antibiotics used in therapy. Candidates should possess a degree/HNC or equivalent in one of the biological sciences. Graduates will be appointed to the Junior Technical Officer scale between £1,533 to £2,199 per annum. Holders of HNC of the equivalent will be appointed to the Technician grade, £1,566 to £2,418 per annum. Experience in antibiotic assay by microbiological and chemical procedures is desirable.—Please apply with details of age, qualifications and experience to Mr P. A. Collingridge, National Institute for Medical Research, The Ridgeway, Mill Hill, NW7 1AA. Tel.: 959 3666. (131)

Battelle

Geneva Research Centre

Seeks urgently

BIOCHEMIST

With previous experience in cell surfaces (immunology, microelectro-phoresis).

—Comprehensive social security scheme
—Four weeks annual vacation
—Assistance with finding accommodation

Applicants should send curriculum vitae, photograph and salary requirements to:—

The Personnel Manager,
Battelle Geneva Research Centre,
7 route de Drize,
1227 Carouge/Geneva, Switzerland. (171)

WESTERN REGIONAL HOSPITAL BOARD

Department of Clinical Physics and Bio-Engineering

Senior Grade Physicist and Electronic Engineer

Applications are invited from graduates with first or second class honours in physics or electrical engineering and at least four years of relevant postgraduate experience for the following posts.

SENIOR PHYSICIST in a team serving the Glasgow Institute of Radiotherapeutics. The Institute's resources include four linear accelerators, neutron generators, computing facilities and a cell-culture laboratory. Special interests include fractionation, metabolic effects of radiation, computing techniques and cellular biology.

SENIOR ELECTRONIC ENGINEER/PHYSICIST in the bioengineering project division undertaking research and development work in cooperation with medical and scientific colleagues in many hospitals. In addition to making a personal contribution of original work the duties include the direction of other graduates and highly qualified technical staff.

Salary scale £2,769 per annum by 6 to £3,591 per annum.

Application forms and job descriptions may be obtained from the Regional Physicist, Western Regional Hospital Board, Department of Clinical Physics and Bio-Engineering, 11 West Graham Street, Glasgow G4 9LF. The last date for applications, Friday January 25, 1974.

(81)

UNIVERSITY OF LEICESTER

READER OR SENIOR LECTURER IN GENETICS

Applications are invited for the post of READER or SENIOR LECTURER in the Department of Genetics. Applicants should have experience in teaching medical students and an interest in human or medical genetics. The person appointed will be responsible for the development of these aspects of genetics in the department consequent upon the foundation of the new medical school, and will be expected to take part in the promotion of developmental medicine in the teaching hospitals. A medical qualification is therefore desirable but not essential.

Level of appointment and initial salary, dependent on qualifications and experience, within the scale £4,368 to £5,496 a year, with F.S.S.U. membership.

Further particulars from the Registrar, to whom applications should be sent by January 28. (74)

UNIVERSITY OF BRITISH COLUMBIA

DEPARTMENT OF CHEMISTRY

Graduate Teaching Assistantships

Graduate Teaching Assistantships are available for candidates with high academic records who wish to pursue further studies towards the M.Sc. and Ph.D. degrees in chemistry. Excellent research facilities are available in all areas of modern chemical science, ranging from bio-inorganic chemistry to chemical physics. Beginning stipends range from $4,350 to $4,550 per annum and depend on the qualifications and seniority of the graduate student. In addition, many special merit awards are made to those Graduate Teaching Assistants who show excellence in the performance of their duties. Numerous graduate scholarships are also available for students with outstanding academic records.

Application forms and further particulars may be obtained from the undersigned.

Professor C. A. McDowell,
Department of Chemistry,
University of British Columbia,
Vancouver, British Columbia,
Canada V6T 1W5. (X2)

L'UNIVERSITE DE MONTREAL

cherche
un

PROFESSEUR D'ECOLOGIE VEGETALE

Qualités requises: Ph.D. ou D.Sc. et expérience de recherche en écologie végétale, de préférence sur les plantes supérieures et avec aspects physiologiques ou génétiques plutôt que phytosociologiques. Possession de la langue française parlée et écrite.

Fonctions: Enseignement de la botanique et plus particulièrement de l'écologie végétale. Recherches. Direction d'étudiants à la maîtrise et au doctorat. Participation à la vie du département.

Titre et traitement: Professeur adjoint, agrégé ou titulaire; de $12,500 à $20,660 par année suivant les qualités du candidat.

Entrée en fonction: Aussitôt que possible.

Date de clôture de réception des candidatures: le 31 mars 1974.

Toute personne intéressée à poser sa candidature est invitée à écrire à:

Professeur Pierre Jolicoeur, Directeur
Département des Sciences biologiques
Université de Montréal
Case postale 6128
Montréal 101, Québec, Canada. (127)

THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY

EXPERIMENTAL OFFICER

(Ref. EEP/10/3)

A temporary Experimental Officer is required in a joint laboratory of Electrical Engineering and Physics to assist in research on the physics of contact electrification.

Applicants, who should hold a degree or similar qualification, should be interested in carrying out experimental work in solid-state physics.

The appointment will be for 2 or 3 years at a salary within the scale £1,569 to £2,889 with F.S.S.U.

Requests for application form, quoting Ref. EEP/10/3, should be sent to: The Registrar, U.M.I.S.T., Sackville Street, Manchester M60 1QD.

Closing date for applications: February 28, 1974. (130)

CELLULAR AND MOLECULAR BIOLOGY

A senior position at the rank of Full or Associate Professor is available in the Department of Biology, York University, Toronto, tenable July 1, 1974. Preferred areas of research interest are: genetics and biochemistry of development, cellular and molecular genetics, neurobiology. Exceptional junior candidates may also be considered. Salary negotiable and commensurate with rank.

Send curriculum vitae, reprints and names of three referees to Professor J. D. Friesen, Department of Biology, York University, 4700 Keele Street, Downsview, Ontario M3J 1[illegible]3, Canada. (114)

MOUNT ALLISON UNIVERSITY

SACKVILLE, N. B., CANADA

The Department of Biology wishes to appoint an ECOLOGIST to its staff, for the academic year 1974–75, to replace a faculty member on sabbatical leave. The appointee will be responsible for two courses: a course on current environmental problems and the man-biosphere relationship, and a course on terrestrial flora and fauna. Opportunities for research. It is expected that the appointee will hold the Ph.D. degree, and have some experience. The appointment can be made at any academic rank, and salary will be commensurate with qualifications and experience.

Applications with curriculum vitae and names of three referees should be sent to Dr C. G. Paterson, Department of Biology, Mount Allison University, Sackville, N. B., Canada. (90)

THE UNIVERSITY OF BIRMINGHAM

INSTITUTE OF CHILD HEALTH

Applications are invited for the post of RESEARCH ASSOCIATE to assist in a 3-year research project to study the relationship between growth hormone, sulphation factor and sex hormones in disorders of growth and puberty in children. The successful applicant would work in the laboratories of the Institute of Child Health, University of Birmingham, under the direction of a Principal Grade Biochemist and Consultant Paediatrician.

Initial salary in the range: £1,569 to £1,785 plus F.S.S.U.

Applications giving details of age, qualifications and experience by January 19, 1974, to Dr. P. H. W. Rayner, Senior Lecturer in Paediatrics, Institute of Child Health, Francis Road, Birmingham B16 8ET. (146)

UNIVERSITY OF LONDON

CHAIR OF CELL BIOLOGY ST. GEORGE'S HOSPITAL MEDICAL SCHOOL

The Senate invite applications for the above Chair. Initial salary to be agreed but not less than £5,625 a year plus £162 London Allowance.

Applications (10 copies) should be received not later than Friday, February 15, 1974, by the Academic Registrar (N), University of London, Senate House, WC1E 7HU, from whom further particulars should first be obtained. (86)

POSITION OPEN

HEAD—DEPARTMENT OF ANATOMY

Applications are invited for the position of head of the department of anatomy at Wayne State University School of Medicine. The position calls for a man or woman having either a Ph.D. or M.D. degree with a strong background in anatomy. Qualified candidates should manifest scholarship in research and teaching along with administrative capacity. The deadline for receipt of applications is April 5, 1974. Starting date and salary are open.

Send letters of inquiry, curriculum vitae, and names of three referees to Dr. Richard S. Berk, Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, MI 48201. We are an equal opportunity employer. (132)

THE OPEN UNIVERSITY

Science Faculty

Technician Grade 4 in Chemistry

Applications are invited for the above post to be taken up as soon as possible. Duties include assistance with research within the discipline, the development of Summer School experiments, demonstrations related to the TV programmes and the home experiment kits. The opportunity also exists to use UV and IR spectrophotometers, gas-liquid chromatograph and a glass vacuum system.

Salary within the scale: £1,848 to £2,163 per annum.

Further particulars may be obtained from the Acting Personnel Manager (CT3), The Open University, P.O. Box 75, Walton Hall, Milton Keynes MK7 6AL. Closing date: Friday, January 25, 1974. (138)

THE UNIVERSITY COLLEGE OF WALES

ABERYSTWYTH

Applications are invited for the post of DIRECTOR of the Welsh Plant Breeding Station, Aberystwyth. The appointment, which will take effect from October 1, 1974, is currently graded as Deputy Chief Scientific Officer in the Agricultural Research Service at a salary within the scale of £6,807 to £7,700 with an allowance of 4½ per cent to offset contributions to F.S.S.U.

The Director will also be a Professor of the University College of Wales, Aberystwyth.

Further particulars can be obtained from the Registrar, to whom applications should be sent by January 31, 1974, together with the names and addresses of three referees. (145)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF CHEMISTRY

LECTURER IN ORGANIC CHEMISTRY

Applications are invited for an appointment as LECTURER IN ORGANIC CHEMISTRY. The salary scale for the lecturer grade ranges from £1,929 to £4,548 per annum, with F.S.S.U., and it is expected that the appointment will be made within the range £1,929 to £2,223.

Forms of application and further details, returnable not later than February 18, 1974, are obtainable from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham. Ref. No. 320. (144)

DEPARTMENT OF BIOLOGY QUEEN'S UNIVERSITY

KINGSTON, ONTARIO

Applications are invited for 3 positions in this department in the general areas (i) vertebrate biology, (ii) cell or developmental biology, and (iii) experimental plant science. Well qualified candidates in related fields may also be considered.

Appointments will be made at the rank of Assistant Professor to start July 1 or September 1, 1974, and will initially be for a period of 1 to 3 years. Successful candidates will be expected to teach comparative anatomy, or introductory cell biology, or plant science (as appropriate), and participate in the graduate and research programmes of the department.

Apply with curriculum vitae to Dr G. R. Wyatt, Head of the Department of Biology, Queen's University, Kingston K7L 3N6, Canada. (118)

TECHNICIAN AND JUNIOR TECHNICIAN required in Department of Clinical Pharmacology to assist in studies on the biochemical properties of cell membranes in relation to receptors for drugs and other biologically active compounds. HNC in Medical Laboratory Science or Applied Biology preferred and previous experience in protein or carbohydrate chemistry advantageous but not essential. Salary according to age, experience and qualifications within scale £708 to £1,395 plus £126 L.W. for Junior Technician and £1,440 to £1,845 plus £126 L.W. for Technician.—Apply, The Secretary, St. Mary's Hospital Medical School, Paddington W2 1PG quoting Ref. CLM 1. (113)

UNIVERSITY OF WINDSOR

DEPARTMENT OF PHYSICS

FACULTY APPOINTMENT IN RELATIVITY

It is hoped to make the appointment at the rank of Assistant Professor or Associate Professor, with duties to begin on July 1, 1974. Preference will be given to applicants with particular qualifications and interests in the theory of relativity and its applications. The successful candidate will be expected to engage in the teaching of undergraduate and postgraduate students, and to participate in the research activities of the Relativistic Group.

Further information may be obtained from Dr L. Krause, Head of the Department. (117)

UNIVERSITY OF GUELPH

DEPARTMENT OF CHEMISTRY

FACULTY POSITION

Applications are invited for a faculty position at the rank of professor, to begin September 1, 1974, to January 1, 1975. Candidates must have a Ph.D. degree in chemistry or biochemistry, a considerable record of research accomplishment in biochemistry or biophysical chemistry, and teaching experience in chemistry or biochemistry. Salary will depend on qualifications and experience. Duties include teaching, supervision of graduate research, and independent research.

Applicants should provide a full curriculum vitae and the names of three referees to A. K. Colter, Chairman, Department of Chemistry, University of Guelph, Guelph, Ontario N1G 2W1, Canada. (115)

UNIVERSITY OF CAMBRIDGE

RESEARCH ASSISTANT

Applications are invited for the position of Graduate Research Assistant from February 1 to July 30, 1974. The work will involve the application of operant techniques to the study of nest-building behaviour in canaries.

Applications including a brief curriculum vitae and the names of two referees should be sent to Dr Joan Stevenson-Hinde, Sub-Department of Animal Behaviour, Madingley, Cambridge CB3 8AA. (110)

ST. BARTHOLOMEW'S HOSPITAL

LONDON, E.C.1

PHYSICS DEPARTMENT

Applications are invited for a post of PRINCIPAL PHYSICIST in the Department of Radiation Physics. The main responsibility of the holder of the post will be to provide a comprehensive clinical physics service to provide the Department of Radiotherapy and to undertake research and developmental work on relevant topics.. Other duties include supervision on the Radiological Protection Service in the Hospital, and consultation on diagnostic X-ray apparatus. Some lecturing in M.Sc. courses and supervision of students reading for higher degrees will also be expected.

Applications, including curriculum vitae, and names of three referees, should be sent to the Personnel Department by January 22, 1974, quoting PT/3874/N. (111)

GUY'S HOSPITAL MEDICAL SCHOOL

Ergonomist or other graduate research assistant with an interest in Biometrics required as soon as possible to join a medical team studying the inter-relationships between work style and back pain. Further particulars and application forms may be obtained from the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT. Applications should be received by January 21, 1974. (168)

CENTRAL MIDDLESEX HOSPITAL

PARK ROYAL, LONDON NW10 7NS

SENIOR TECHNICIAN/TECHNICIAN required for department of Chemical Pathology. For further details and visit to department telephone Chief Technician, 01-965 5733, Ext. 551. (166)

MCMASTER UNIVERSITY

HAMILTON, ONTARIO, CANADA

DEPARTMENT OF APPLIED MATHEMATICS

Applications are invited for one or more appointments at Assistant Professor level, possibly higher rank. Areas: Appl. Math., O.R., or Comp. Sci. Ph.D. required, previous teaching experience desirable. Starting date: July 1, 1973. 1973/74 minimum salary level: Assistant Professor $12,000. Applicants to send curriculum vitae, details of research and teaching experience, list of publications, and names of 3 referees, to Dr. G. Field, Chairman, Applied Mathematics Department, McMaster University, Hamilton, Ontario, L8S4K1, from whom further details may be obtained. (151)

POSTDOCTORAL RESEARCH IN IMMUNOLOGY

A position is open for research on cell-mediated immunity in relation to cancer. Recent Ph.D. preferred.

Send curriculum vitae to: Dr. E. Sabbadini, Department of Immunology, University of Manitoba, 795 McDermot Avenue, Winnipeg, Manitoba, Canada R3E 0W3. (152)

THE KENNEDY INSTITUTE

IMMUNOLOGY DIVISION

RESEARCH TECHNICIAN required in the Laboratory of Immunochemistry for work on antibody affinity under the direction of Dr. M. Steward. Minimum qualifications Higher National Certificate or equivalent, preferably with experience of biochemical or immunological techniques. Written applications, naming two referees, to: the Laboratory Superintendent, The Kennedy Institute, Bute Gardens, Hammersmith, W6 7DW. (170)

TECHNICIAN required to assist with original biochemical and immunological research on cancer. Experience in a relevant subject is desirable but not essential. The successful candidate will be appointed on a salary scale of £1,440 to £2,292.—Apply enclosing a curriculum vitae and the names of two referees to: Dr G. Wiernik, Research Institute, Churchill Hospital, Headington, Oxford. (91)

SENIOR HARVARD CHAIR in the School of Public Health, the John Rock Professor of Population Sciences is to be filled by a scholar specialising in population. Harvard is an equal opportunity employer. To be considered please write Dr Howard H. Hiatt, Dean, Harvard School of Public Health, 55 Shattuck Street, Boston, Massachusetts 02115. (83)

ABSTRACTOR

A graduate in zoology is required for full-time abstracting work. A knowledge of a foreign language is an advantage. Please apply to Dr. E. S. Krudy, Information Retrieval Ltd., 1 Falconberg Court, London W1V 5FG. (169)

DEPARTMENT OF CHEMISTRY

UNIVERSITY OF LEICESTER

A TECHNICIAN (Grade 6) is required to take responsibility for the Inorganic, Organic and Physical Chemistry Teaching Laboratories. Salary £2,370 to £2,874 per annum.

Apply, giving full details of experience, qualifications and the names of two referees to Mr H. T. Whitt. (128)

BIO-MEDICAL GRADUATE

Major bio-medical publishing company requires recent graduate to assist the Marketing Director in the world-wide promotion and sale of scientific periodicals. Candidates must be prepared after training to travel. Offices in Central London. Initially send curriculum vitae with current address and telephone number to A. J. Woodcock, Information Retrieval Limited, 1 Falconberg Court, London W1V 5FG. (164)

LABORATORY TECHNICIAN GRADE 3 REquired to assist in the Electron Microscope Unit. "A" level or equivalent standard required. Experience desirable but not essential as full training given. Salary in range £1,650 by £54 to £1,920 per annum plus £175 per annum London Weighting. Five-day week. Five weeks (in total) annual leave. Letters only to Assistant Secretary (Establishment) (EMU/T), Queen Mary College, Mile End Road, London E1 4NS, stating age, experience and qualifications. (140)

SYMPOSIUM

SOCIETY OF CHEMICAL INDUSTRY

LONDON SECTION

A Symposium on CATALYSIS AND INHIBITION OF OXIDATION PROCESSES, arranged by Professor N. URI, will be held at THE CITY UNIVERSITY, St John Street, London EC1V 4PB, on Monday, March 4, 1974.

PROGRAMME: 9.15 a.m., Opening of the Symposium, Dr R. M. Evans, Chairman, London Section. 9.20 a.m. to 10.45 a.m., General Principles of Catalytic Oxidation, Dr. R. J. Sampson (I.C.I. Petrochemicals Division). 10.45 a.m. to 11.45 a.m., Catalytic Oxidation of Olefins on Metals and on Certain Metal Ketonides, Professor D. Bryce-Smith (Reading University). 11.45 a.m. to 12.45 p.m., Use of Noble Metals in Catalytic Oxidation, Dr G. J. K. Acres (Johnson Matthey Ltd). 12.45 p.m. to 2.00 p.m., Lunch. 2.00 p.m. to 3.00 p.m., Use of Antioxidants in the Food Industry, Mr K. G. Berger (J. Lyons and Co). 3.00 p.m. to 4.00 p.m., Thermal and Photochemical Oxidation of Polymers and its Prevention, Professor N. Uri (E.R.D.E. and The City University). 4.25 p.m. to 5.25 p.m., Catalysis and Inhibition of Paint Drying, Mr J. Turner (Hardman and Holden Ltd).

FEES: £3 for members of S.C.I. (inclusive of lunch); £5 for non-members (inclusive of lunch); £3 for the Symposium Dinner.

Registration forms can be obtained from Dr R. F. Greenwood, Hon. Secretary, London Section, Chemistry Department, The City University, St John Street, London EC1V 4PB. Telephone 01-253 4399. (175)

GRANTS & SCHOLARSHIPS

DUBLIN INSTITUTE FOR ADVANCED STUDIES

SCHOOL OF THEORETICAL PHYSICS

The Governing Board of the School of Theoretical Physics invites applications for RESEARCH SCHOLARSHIPS tenable in the School during the academic year 1974-75. A limited number of stipends will be available. These awards are normally made at postdoctoral level.

For further particulars and Forms of Application candidates should apply immediately to The Registrar, Dublin Institute for Advanced Studies, 10 Burlington Road, Dublin 4, Ireland. Completed applications should reach the Registrar on or before April 1, 1974. (161)

Please
mention
this Journal
when
answering
advertisements

FELLOWSHIPS AND STUDENTSHIPS

UNIVERSITY OF MELBOURNE

SENIOR RESEARCH FELLOWSHIP

The University of Melbourne invites applications for a Senior Research Fellowship tenable for one year but renewable in special circumstances for a second. The Fellowship is intended for a scholar with considerable research experience, e.g., a senior member of academic staff who may be on sabbatical leave from his own university. The salary will be in the range of \$A12,268 and \$A15,187 and up to \$A1,000 may be paid towards the cost of transport.

Applications should be lodged with the Office of the Dean of Graduate Studies, University of Melbourne, Parkville, 3052, Victoria, Australia, by **February 28, 1974.**

Conditions for the Fellowship may be obtained from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WO1H 0PF or from the undersigned.

R. A. CUMMING,
Secretary for Graduate Studies.
(100)

BRITISH MUSEUM (NATURAL HISTORY)

POSTGRADUATE RESEARCH STUDENTSHIPS 1974

Two studentships will be awarded, tenable for 3 years at the British Museum (Natural History) and commencing October 1974. Arrangements will be made with an appropriate university for higher degree registration and joint supervision of the research project.

The research areas for which applications are invited are as follows:

Department of Zoology

Biometric analysis of mammalian remains in relation to animal resources in Tudor England.

Department of Entomology

1. Cytotaxonomy of selected British Simuliidae (Black-flies, Diptera).
2. Biometric analysis of the Potato-Aphid species group.

There are good research facilities available including those for scanning and transmission electron microscopy, serology, biochemistry and biometrics.

Eligibility: Applicants to be British and normally resident in Great Britain, aged 27 or less and having a first or upper-second-class honours degree; students graduating in 1974 can be offered awards subject to their degree results reaching this level.

Students interested in applying should write to the Secretary (Postgraduate Studentships), British Museum (Natural History), Cromwell Road, London SW7 5BD, for application forms and conditions of the award. **Closing date for applications March 15, 1974.** (94)

UNIVERSITY OF WARWICK

POSTDOCTORAL FELLOWSHIP IN BIOLOGICAL SCIENCES

Applications are invited for the above post to work on the biochemistry of virus RNA replication. The post would be suitable for a biochemist with an interest in animal virology or a virologist with a good knowledge of biochemistry. Experience of working with nucleic acids or their polymerases would be an advantage. The appointment is for nineteen months commencing March 1, 1974. Salary in the range £1,929, £2,058, £2,223 per annum with F.S.S.U.

Further details and application forms may be obtained from the Academic Registrar, University of Warwick, Coventry CV4 7AL, to whom completed forms should be returned by January 25, 1974, quoting Ref. No. 20/Q/73. (80)

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DEPARTMENTS OF CHEMISTRY AND MICROBIOLOGY

A POSTDOCTORAL FELLOWSHIP

(Salary range: £1,929 to £2,223)

is available to work on the effect of metal/organic compounds on microbial growth reproduction and differentiation under the joint direction of Professor R. D. Gillard (Chemistry) and Professor D. E. Hughes (Microbiology).

Duties to commence as soon as possible. Closing date for applications is two weeks from the appearance of this advertisement.

Applications, together with the names and addresses of two referees, should be forwarded to the Registrar, University College, P.O. Box 78, Cardiff CF1 1XL, from whom further particulars will be available. Please quote reference 0973. (84)

European Molecular Biology Organization

(EMBO)

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The European Molecular Biology Organization intends to award to scientists working in laboratories within the European area both short-term (from a few days to several weeks) and longer-term fellowships for collaborative research or advanced training in molecular biology.

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Application forms and further details may be obtained from Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, 6900 Heidelberg 1, Postfach 2029 (Postfach 1022.40 after July 1, 1974), Germany.

Application for Short-term Fellowships may be made at any time and will be decided upon soon after receipt of the application. Applications for Long-term Fellowships should be made before February 1 for a decision by April 30 and before August 1 for a decision before October 31. Because the selection procedure involves an interview these deadlines for application should be respected.

(X4)

Ministry of Agriculture, Fisheries and Food

JOINT AGRICULTURAL DEVELOPMENT AND ADVISORY SERVICE/
AGRICULTURAL RESEARCH COUNCIL
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DUTIES

2. The appointee will be a member of a research team investigating crop tolerances to transient waterlogging with a view to determining minimum drainage requirements and will have the opportunity of gaining experience of a wide range of plant physiological and physical methods for studying the performance of plant root systems in soils.

QUALIFICATIONS

3. A good honours degree in one of the Agricultural Sciences and at least 2 years postgraduate research experience.

REMUNERATION

4. The value of the Junior Research Fellowship is fixed for the duration at the time of appointment in the following range:

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APPLICATION

5. Application forms may be obtained from Mr. J. Barnes, Personnel Division IIA Ministry of Agriculture, Fisheries and Food, Horseferry Road, London SW1. (01-834 8511 Ext. 6237.)

Closing date for completed applications February 1, 1974.

(143)

UNIVERSITY OF BRISTOL

The Department of Bacteriology has a vacancy for a two-year research studentship to investigate the fate of bacteria from sewage entering the Bristol Channel. The appointee will be expected to register for a higher degree and must therefore have a 1st or 2A honours degree. Studentship £825 per annum.

Applications, together with the names of two referees, should be sent to Professor M. H. Richmond, Department of Bacteriology, Medical School, University Walk, Bristol BS8 1TD.

(121)

UNIVERSITY OF SYDNEY

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Applications are invited for a post-doctoral Research Fellowship in the area of protein crystallography. The appointee will participate in a research project on the structure analysis of a metalloprotein, in collaboration with a group which includes crystallographers, biochemists, and co-ordination chemists. Experience in protein crystal structure analysis will be an advantage. Excellent instrumental and computational facilities exist.

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Applications, including details of experience and qualifications and names of two referees by February 8, 1974, to the Registrar, University of Sydney, N.S.W. 2006, Australia, from whom further information is available. Further details from Professor H. C. Freeman, Department of Inorganic Chemistry.

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LECTURES AND COURSES

THE FOURTH EUROPEAN WORKSHOP ON DRUG METABOLISM

The Fourth European Workshop on Drug Metabolism will be held at the University of Mainz, West Germany, from September 15-20, 1974.

For further information write to: Professor Dr. K. J. Netter, The Fourth European Workshop on Drug Metabolism, Pharmakologische Institut der Universitat Mainz, Langenbeckstrasse 1, 6500 Mainz, West Germany.

(96)

UNIVERSITY OF LONDON: A LECTURE entitled "Neutrinos as Probes of Nucleon Structure" will be delivered by Professor D. H. Perkins (Oxford) at 5.30 p.m. on January 14, at University College (Physics Lecture Theatre), Gower Street, W.C.1. Admission free, without ticket.—Academic Registrar.

(153)

UNIVERSITY OF LONDON: A COURSE OF two lectures entitled "1. Geographical Aspects of Structural Change. The Case of Sweden. 2. A Review of Historico-Geographical Research in Sweden" will be delivered by Professor Staffan Helmfrid (Stockholm) at 5.30 p.m. on January 15 and 16 at University College (Engineering Lecture Theatre), Gower Street, W.C.1. Admission free, without ticket.—Academic Registrar. (154)

UNIVERSITY OF LONDON: A COURSE OF two lectures entitled "Aromaticity and Pericyclic Reactions" will be delivered by Professor M. J. S. Dewar (Texas) at 4.30 p.m. on January 15 and 17 at Queen Mary College, Mile End Road, E.1. Admission free, without ticket.—Academic Registrar.

(155)

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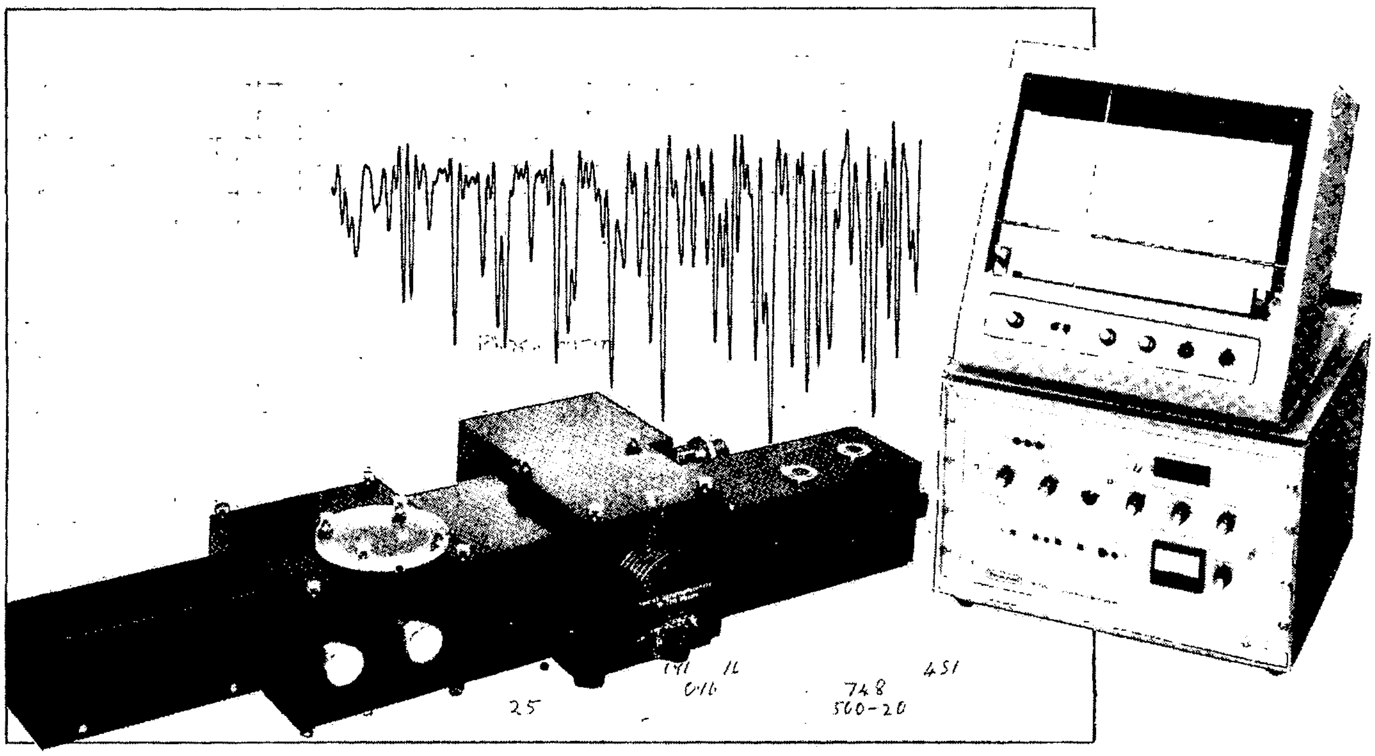

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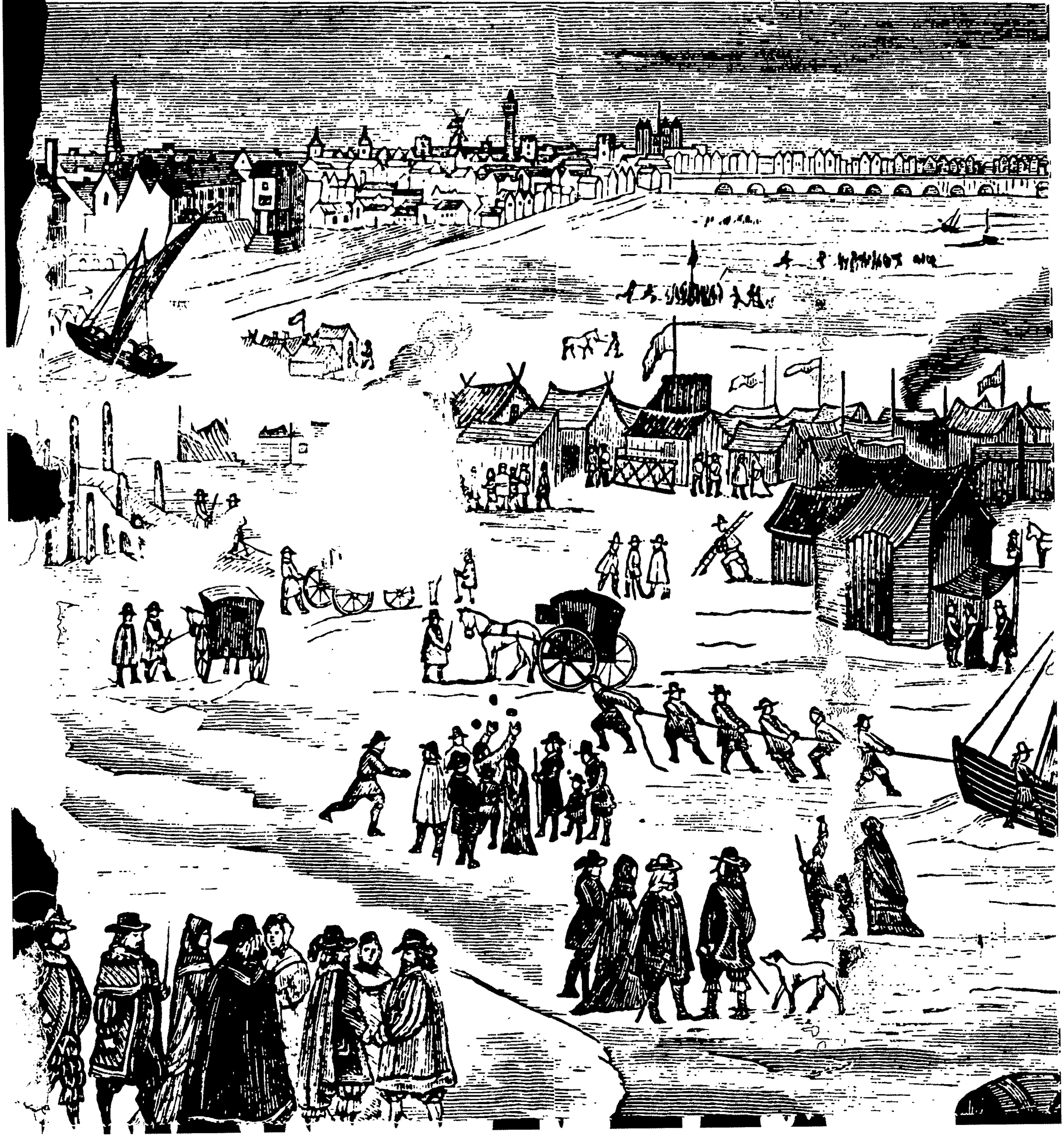

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Engraving of frost fair on the Thames, 1683–84 (copyright Radio Times Hulton Picture Library), which last froze in 1813–14. Climatic changes are discussed on pages 127 and 131.

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Precision Instruments

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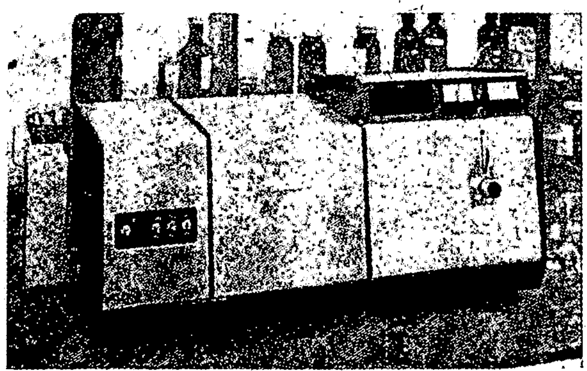

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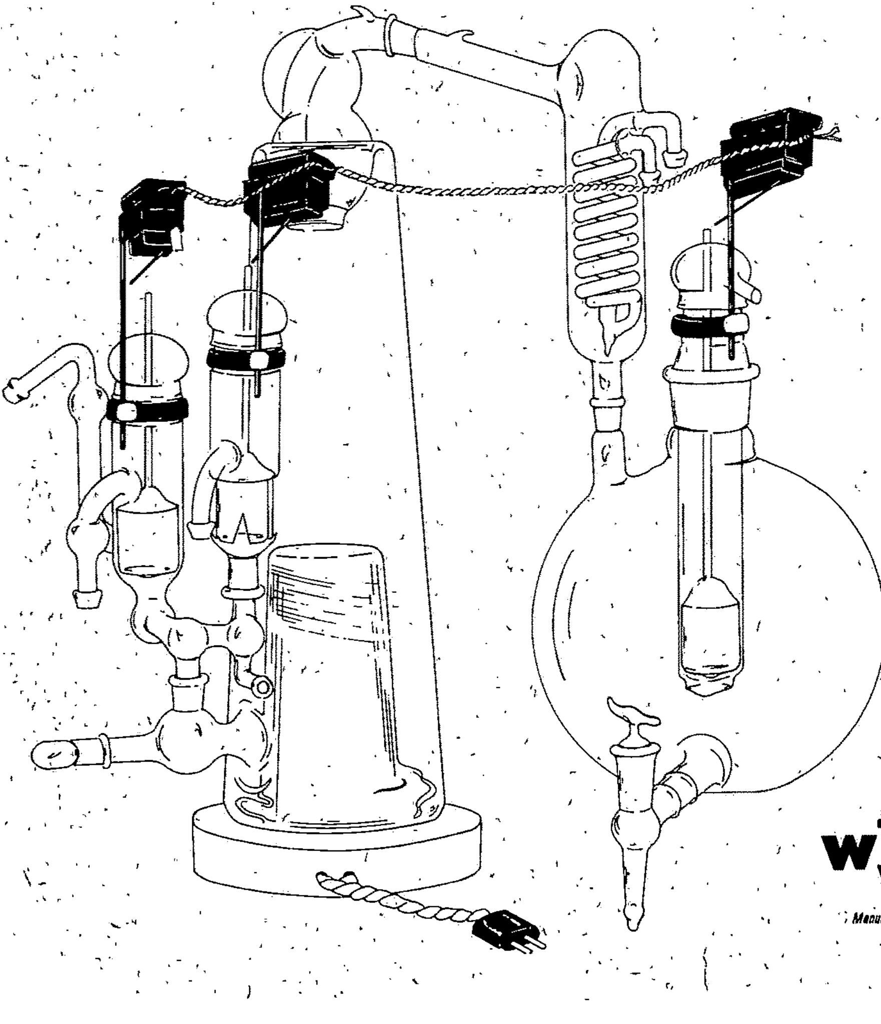

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A fuller guide appeared in *Nature* (**246**, 238; 1973).

Because of the imposition of a three-day working week in Britain, *Nature* is being printed in the United States by the William Byrd Press in Richmond, Virginia, for the duration of the emergency. This issue is the first to be printed in the United States, and it has been made up of material set partly in England and partly in the United States. Every effort is being made to ensure that subscribers receive their copies at the normal time.

The London office continues to be open five days a week, and manuscripts are being handled there normally. Subscriptions are also still being dealt with at the Subscriptions Department in Basingstoke, England.

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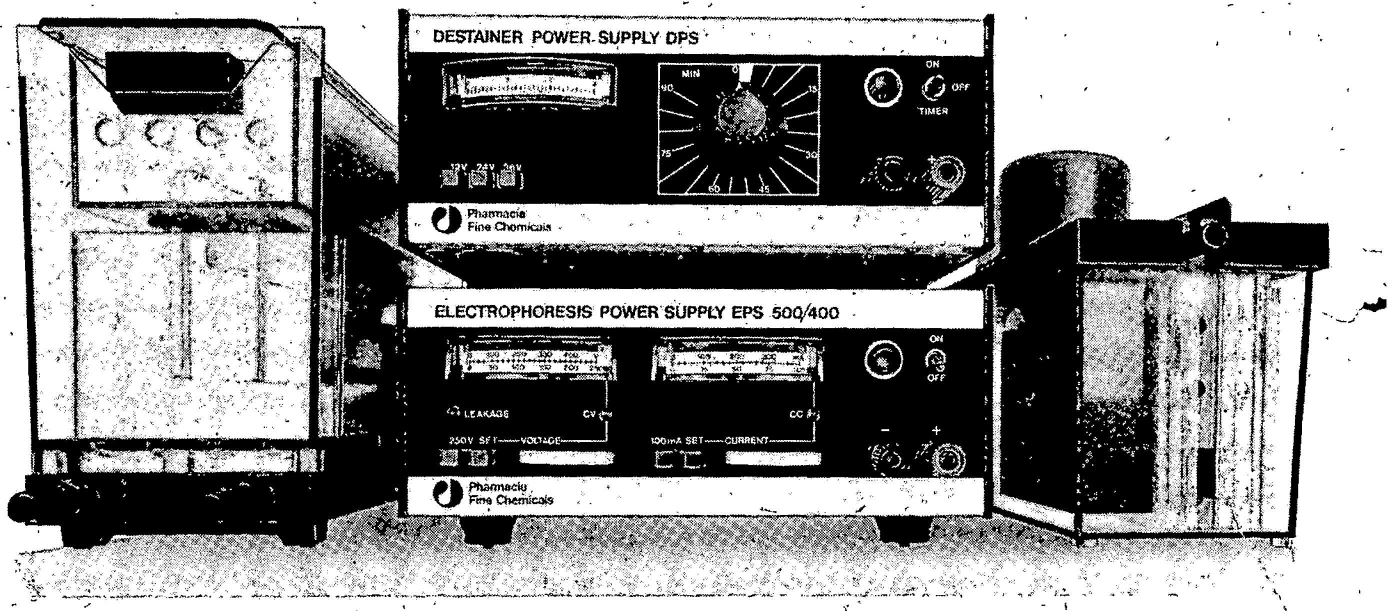

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Research and energy independence

DURING the past year, the Nixon Administration has done a rather spectacular U-turn in its public statements on energy research and development. Back in April 1973, when President Nixon was still referring to the looming energy shortages as a challenge rather than a crisis, he said "it is foolish and self-defeating to allocate funds (to energy research and development) more rapidly than they can be effectively spent". But in June he found himself not only promising to spend an extra $100 million this year, but also announcing that this would be followed by a five-year energy research and development programme which will begin in 1975 and which will eventually soak up $10,000 million in federal funds. Finally, in November, the President was claiming that this technological effort, coupled with conservation measures, could win independence for the United States from foreign suppliers of oil by 1980.

Welcome as this late espousal of the technological answer may be—particularly for those directly engaged in such research and development—there are nevertheless, strong doubts about whether such a research and development programme will be sufficient to ensure independence. And there are also questions—based as much on the structure of the federal government itself as on the Administration's poor track record in supporting science and technology—about the endurance of the commitment to energy research.

The Administration's research and development effort is likely to follow a set of proposals drawn up by the Chairman of the Atomic Energy Commission at President Nixon's request, and delivered to the White House in December (see *Nature*, **246**, 370; 1973). The proposals, which conformed to President Nixon's suggested ceiling of $10,000 million for a five-year programme, call for massive concentration on short term goals, such as more effective use of coal and energy conservation, and they also call for almost half the money to be spent on nuclear research and development.

The first thing to be said is that Presidential claims notwithstanding, the suggestion that the United States will be self sufficient in energy by 1980 is nonsense. Even with the $10,000 million programme, the AEC report suggests that the United States will in fact be importing more oil in 1980 than at present (5.9 million barrels, compared with 5.1 million in 1972). 1985 is the earliest date by which independence can be achieved, the report says, and even that prediction is based on some dubious assumptions.

For one thing, the Administration's plan tacitly assumes that once the federal government has done the research and development and proven the feasibility of energy technologies, private industry will quickly step in and provide the capital necessary to exploit it. Certainly, the AEC's plan calls for some joint industry–government collaboration in setting up pilot plants, but even the Chairman of the Atomic Energy Commission, Dr Dixie Lee Ray, reckoned at a meeting of the President's Energy R&D Advisory Council in December that it will cost as much as $50,000 million to exploit the fruits of the research and development programme.

A better approach to buying independence for the United States is contained in a bill sponsored chiefly by Senator Henry M. Jackson, which was passed by the Senate at about the same time as the AEC report was sent to the White House. Not only does that bill provide much more money—Jackson reckons as much as $15,000 million on all energy research and development would be spent between 1975 and 1979 if his bill is adopted—but it also contains an important provision authorising the establishment of joint government–industry development programmes. These would provide federal funds directly for the construction of demonstration plants to exploit new energy technologies and would provide a measure of government control over their operation. That bill is now awaiting action in the House of Representatives, where it demands careful consideration. But even if it survives the Congressional circuit, there is still no guarantee that the money would be spent, for it would then have to go through the appropriations route, and the Administration would also have to be persuaded to spend the money.

100 years ago

IS England rapidly losing that commercial and manufacturing supremacy which she has held before all the world for generations past? Is she going the way of Venice, of Florence, of Holland? If so, is it because she feels blindly secure that "what hath been, will be," neglecting the means on which success in commerce and manufactures in these days depends—means which are being so industriously used by rival nations, that they are rapidly shooting ahead of England on England's own ground?

From *Nature*, **9**, 217, January 22, 1873.

international news

Kohoutek makes a good night out

John Hall

ITS complex molecules apart, there is no doubt that the biggest let-down of the astronomical year was the arrival of the hapless Dr Kohoutek's comet. After nine months of speculation and counter speculation about the size and brightness of this spectacle it is now possible to make a single dogmatic assertion on the subject without fear of contradiction: that distance lent enchantment to the eye. The thing is not going to bowl over anybody on account of its splendour, and the complex molecules cannot be detected with binoculars.

Turning a blind eye to the dim reality of the phenomenon, at least two British companies had the enterprise to cash in on Kohoutek. Kohoutek Ltd settled for comet 'T' shirts and a million shares at a penny a go, and Transolar Travel plumped for an observation flight, price £12 to £24 a head, which pulled in 140 enthusiasts, culled largely from the ranks of amateur astronomy societies. The media were there to observe the observers and, curiously for an event which was to be enjoyed at a distance of 90 million miles, clutches of spotters milled about with portable tape recorders and instamatic cameras topped with flash cubes which could scarcely have thrown much light on the subject. The British astronomer Mr Patrick Moore was clearly visible to the naked eye.

The tone of the expedition was set at a preliminary lecture, at which a director of Transolar projected an artist's impression of a nineteenth century comet, about as bright as a full moon and stretching half across the sky, which he confessed to be much the sort of show he expected from Kohoutek. This was greeted by polite laughter from the spotters who throughout the lecture insisted on photographing the lecturer, the slides, each other, and such aeroplanes as passed within range.

Once above the Gatwick clouds in a couple of BAC 1-11s, an excited babbling broke out as Venus and Jupiter hove into view, and a gentleman from the BBC paced the gangway soliloquising about the agitation. Then from the flight deck an announcement was made that, at 33,000 feet, the comet was no longer obscured by cloud, and it was all going to be worthwhile. Patrick Moore raised his voice above all others to issue directions for identifying the target ("left from Venus to Jupiter, then up a bit") and was heavily photographed for his pains.

Then, sure enough, we were able to see a distinct sort of smudge, with a tail extending five degrees maybe, representing perhaps six and a half million miles, and of magnitude three and a half, possibly. Certainly it was a distinct sort of smudge. More plainly, it was possible to make out the street lighting of Wales, Wallasey and the shape of the Mersey estuary, then the Isle of Man, Manchester and, more controversially, Leeds.

The astronomers passed among each other cassette recorders, making essays in historical audio verité. "Have you seen the comet?" "Yes, I've seen the comet. It has a tail extending five degrees maybe, representing perhaps six and a half million . . ." and so on. Finally, since there was only one window to every two observers, those who did not have the good fortune to be twisting their necks and spines to crane after the comet, insisted on paying their respects to the captain, since a better view could be had from the flight deck. A visiting rota had to be arranged when the astronomers, firing their flash cubes as they went, were thought to be harassing the pilot. "It's all very well, but we just cannot have people sitting on the captain's shoulders", the Transolar man remonstrated.

Patrick Moore initiated a round of rousing applause for the organisers, who had put on the entire junket with nothing to gain but hard cash, and the only note of concern was voiced when we passed, on the Lancashire coast, a bright flame of petrochemical waste which, I commented, looked remarkably like the exhaust of a SAM-7 missile.

Nuclear safety

Colin Norman, Washington

THE longstanding and bitter dispute about the safety of American light water reactors is about to boil up again in the United States. Next week, the Joint Committee on Atomic Energy is planning to hold three days of public hearings which will put the spotlight on perhaps the most contentious nuclear safety issue: the emergency cooling system which is supposed to flood the reactor core with water if an accident suddenly robs the reactor of coolant. Arguments about whether or not this piece of hardware would actually work in an emergency have been batted back and forth between the Atomic Energy Commission and its critics for the past two or three years. Since the British Government may soon put in an order for American reactors, the matter is of more than parochial interest.

Next week's hearings, which will take place on January 22–24, will be concerned chiefly with a number of regulations issued by the AEC at the end of December. Based on a marathon public inquiry into the functioning of the emergency core cooling system (ECCS), held by the AEC over a four-month period last year, the regulations will reduce the peak operating temperature of light water reactors by 100°F, and put limits on the amount of oxidation permitted in the fuel cladding.

According to officials of the Atomic Energy Commission, the regulations will reduce the power output from some reactors, and provide an adequate margin of safety in the (unlikely) event of loss of coolant. According to the critics, however, they do no such thing, since the regulations are merely cosmetic changes designed mostly for public relations purposes.

The hearings will also take place against a backdrop of persistent rumours of palace revolts and power struggles among top AEC officials. According to some accounts, open warfare has broken out between Dr Dixie Lee Ray, the Chairman of the AEC, and some of the other commissioners. A number of key officials have resigned from the agency in the past few months, including Mr Milton Shaw, director of the Division of Reactor Technology, Mr Robert E. Hollingsworth, the AEC's General Manager, and Alvin M. Weinberg, Director of the Oak Ridge Laboratory. Moreover, James T. Ramey, a former general counsel of the Joint Committee on Atomic Energy, was reappointed to the Commission last year after his term of office expired. Since Ramey provided a link between the AEC and its Congressional watchdog, his loss has stirred up considerable animosity between Dr Ray and some committee members.

Scientists are playing their part in power saving

John Hall

IN Britain the arrival of the three-day working week, complete with regulations restricting the use of power, raises the spectre of an earlier age of science. Might one expect, without undue romanticism, to be conducting enquiries by the glow of a tallow candle? The speculation, as it happens, turns out to be as inaccurate as it is morbid. By and large, research goes ahead under neon lights, albeit fewer of them, and with power supplies reduced in some cases 60% of normal and in others not at

The Statutory Order restricting the use of electricity offers three channels of escape (at least) which ensure the survival of research programmes whose existence depends on supplies of power. In the first place, work which is taking place at a university is in the clear because of the exemption from cuts allowed to places of education. A further clause in the order allows the operation of apparatus "which has been continuously in use for purposes of research until such time as the operation of the said apparatus can be discontinued without causing loss or damage"—a flexible-looking provision, since the order does not actually specify that the loss should be of materials rather than of information. And additionally, the regular supply of power is allowed for the operation of any computer "and any other equipment necessary for the proper functioning thereof". As a last resort a laboratory can make a special plea to regional officers of the Department of Trade and Industry, who have been instructed to avoid "rigid attitudes" in dealing with such applications.

At Jodrell Bank, in spite of a power reduction of 75%, the radio telescopes are still functioning twenty-four hours a day for seven days a week. In fact, says Professor Sir Bernard Lovell, the telescopes use less power than the cooker in the station's canteen. The total load for the entire establishment at present is only 35 kW and even if the regulations were changed in the event of a worsening power situation, Jodrell Bank's own generator could fill the gap for several weeks. The Appleton Laboratory, formerly the Radio and Space Research Station, at Slough is working a five-day week with only three days of power and has had to delay work on certain projects.

University College, London, which hopes to take its electricity for the full five days, but at a voluntarily reduced level of 60% of normal consumption, also has certain difficulties. As it happens, the Faculty of Medical Sciences has no animal experiments in train and since its other projects were part of teaching programmes, none of its work has been affected. The Department of Chemical Engineering, with a pilot plant which attempts to translate laboratory biochemistry into industrial process design, is, however, a heavy user of power. Large scale fermentation and isolation runs, carried out under industrial conditions, can use motor power amounting to 80 horse power.

Dr Peter Dunnill says the department can scarcely afford to have its £0.25 million worth of equipment standing idle but at the same time it is not really feasible to do biological research on a three-day week or at reduced power. Rather, the department will try to take its 60% of power at the normal rate for, say, one full week in every four.

The Royal Aircraft Establishment at Farnborough, reduced to 65% consumption, spread over five days a week, has elected, stoically, to "make do and mend". Computer work and continous process research is unaffected but testing programmes, including work for Concorde, has to be done "as and when we can manage it", said a spokesman. The nearby National Gas Turbine Establishment is working on the same basis.

The Atomic Energy Authority's research establishments at Culham and Harwell are surviving by using 65% of their November daily average requirement, as are the Dounreay Fast Reactor and the Winfrith Steam Generating Heavy Water Reactor—in spite of the fact that they generate 114 MW between them. Apparently it is considered unethical to plug in your lighting and heating at source.

Naples at the crossroads

THERE is great international interest in the establishment of new conditions at the Naples Zoological Station. The station fulfils for marine biology (along with a number of other stations such as Monaco, Plymouth and Woods Hole) similar functions to those that large accelerators do for nuclear physics. In addition to having a staff of its own, the station is capable of housing large numbers of international visitors. It is not alone among Italian scientific institutions in having recently fallen on hard times as a result of dissatisfaction with the administration among its resident workers.

The Italian government has reacted to discontent within the station by installing a Commissario whilst the administration was overhauled. A new set of statutes has been provided and the financial basis for the station is much sounder. Italy will provide about £400,000 annually and any country which contributes more than £40,000 annually will be entitled to a seat on the new Administrative Council. It is hoped that Britain will have a seat.

Prospects are much brighter for a settled future but the remaining question is whether the science will be stimulating. This hangs on two issues—who will be the new director and what sort of programmes will he institute? The permanent staff have gone to unusual lengths to ensure that the community is well informed of the needs of the station; a recent advertisement in *Nature* invited suggestions for the development of the institution and by inference nominations for the directorship. It is important at this juncture that, even though the station is understaffed, the decision not be made in a hurry—certainly not until the council has had time to feel its feet.

PSAC reports from the grave

Colin Norman, Washington

THE President's Science Advisory Committee (PSAC) has posthumously complained about the federal government's methods of regulating public exposure to chemicals in the United States. It has also added its ghostly voice to the chorus of opposition to one of the most controversial, and certainly the most effective, pieces of consumer legislation on the lawbooks—the so-called Delaney Amendment which seeks to ban the addition to food of any substance which has been found to cause cancer in animals.

Although PSAC was a victim of President Nixon's post-election massacre of the science advisory apparatus in the United States (the massacre which scrapped the Office of Science and Technology and banished scientists from the White House), its reports continue to surface in Washington as reminders of a past age in science policy making. This latest PSAC report (*Chemicals and Health*, US Government Printing Office, $2.75), was released last week by Dr H. Guyford Stever, Director of the National Science Foundation, and inheritor of most of the responsibilities which were assigned to the Office of Science and Technology. Stever described the report last week as a "broad, yet intensive, examination of the processes of Federal regulation of chemicals . . . performed in the name of health".

Prepared by a committee which met under the chairmanship of Dr John W. Tukey, Professor of Statistics at Princeton, the report argues for more flexibility in regulatory decision-making: in other words, for risks and benefits to be properly weighed before a chemical is banned or restricted. The committee also argues for more public disclosure of the principles behind regulatory decisions, for public discussion of issues as they arise, and for independent advisory committees to share the burden of regulation with government administrators. Like every other committee which has reported on the matter (and there have been several over the past decade), the PSAC committee also calls for more research into the health effects of environmental chemicals. In particular, it calls for a greater role in environmental research to be assigned to the National Institute of Environmental Health Sciences.

The committee reckons that one of its most important recommendations is that the Administrator of the Food and Drug Administration and the head of the Environmental Protection Agency should each have an advisory board to assist in major regulatory decisions. Such boards would consist of two classes of members: some would be appointed by the administrator from a list suggested by the National Academy of Sciences, and the rest would be drawn from the general public and appointed by the President. The idea would be for the boards to prepare reports and analyses at the administrator's request, and they would also be able to produce reports on their own initiative. Such an arrangement would be analogous in some respects to the Committee on the Safety of Drugs in the United Kingdom, except for the fact that the boards' reports would be made public—a far cry from the obsessive secrecy that surrounds the work of the British committee.

The PSAC report also comes up with the usual recommendations for increasing public participation in, and knowledge of, regulatory issues and decisions. It also makes the obvious—though frequently overlooked—point that the most widespread and damaging effects of exposure to chemicals come from the consumption of alcohol, tobacco and drugs. Programmes designed to protect public health should thus be directed chiefly at reducing consumption of those chemicals, the committee argues.

A more controversial part of the report is concerned with the Delaney Amendment. Although Dr Tukey said last week that the committee does not specifically disapprove of the Delaney Amendment, it is clear that the amendment is not exactly regarded as the epitome of good regulatory policy. What the committee chiefly objects to is the inflexibility that the clause imposes on the administrator of the Food and Drug Agency.

The amendment, which takes its name from John Delaney, a New York Congressman who is noted for little else, imposes an absolute ban on adding any chemical to food if it has been found to be carcinogenic to animals. The clause has always irked many food manufacturers, because it has led to the banning of chemicals on the basis of what they consider to be unrealistic tests. On the other hand, many cancer scientists have supported the clause because they argue that it should be government policy to reduce public exposure to carcinogens as much as possible. The PSAC committee adopts the position that the Administrator of FDA should have the ability to set safe levels of chemicals in food, on the basis of dose-response studies.

Publication of the report is timely, not only because some of the fiercest and most bitter battles between the federal government and the public continue to take place over the regulation of food additives, drugs and other chemicals in the environment, but also because the energy crisis is forcing the government to backtrack on environmental laws which are designed to protect people against harmful pollutants. Moreover, some trade unions in the United States are taking an increasingly keen interest in the exposure of workers to chemicals in the workplace. The Chemical and Atomic Workers Union, for example, recently held a long strike against the Shell Oil Company over working conditions, in which questions of exposure of workers to carcinogenic chemicals played a large part. Such disputes are clearly going to increase.

Soyuz-13

From our Soviet Correspondent

THE programme of the Soyuz-13 flight (December 18-26, 1973) seems to have been a multipurpose one, combining routine tests of the hardware, astrophysical and geophysical observations, and preparations for possible long term space flights in the future.

The emphasis given to further checks of the on-board systems, to new methods of manual and automatic control, and to autonomic navigation systems, seems at least partly aimed at maintaining the confidence of the United States participants in the forthcoming Soyuz-Apollo linkups. Indeed, one new navigation project involving observations and photographs of luminous effects before sunrise and after sunset, beyond the line of the horizon, can only be relevant for such orbital missions.

The medical checks, which include the new Levkaya apparatus to monitor cerebral circulation during weightlessness, in conditions of rest, and also after graduated exercise with a special expander with a pull of 30 kg at a rate of thirty times a minute, may also be directed in the first instance towards the joint project, although such data will obviously be of wide application in space medicine.

Similarly, for home consumption (for undercurrents of discontent at the cost of the Soviet space programme still emerge from time to time, mainly in the form of black humour), considerable press coverage has been given to the spectroscopic investigations of earth resources and pollution—now dignified by the name "cosmic ecology". Although this is one of the routine tasks of the Kosmos satellites, it is difficult to see the cost effectiveness of using a manned station for the purpose.

The most interesting feature, however, is that this short term flight has taken over two projects first envisa for the Salyut station, one astrophys and one biological. The Orion II telesc (an improved version of the Orion carried by Salyut) was used to carry out extensive ultraviolet spectroscopy of the Sun and stars. The new system includes a wide-field meniscus telescope capable of ultraviolet photography of stars down to magnitude 9.5 or 10.

The other experiment taken over from Salyut is the Oasis enclosed environment recycling system. Oasis originally contained higher plants such as flax and Peking cabbage. The new version, Oasis II, is a microorganism system which consists of two interconnected cylinders. The first cylinder contains bacteria which feed on hydrogen produced by electrolysis, whereas the oth[illegible] contains bacteria which break down urea, absorbing oxygen generated in the first cylinder and liberating carbon dioxide which is then used by the bacteria in the first cylinder. Although only a small scale version has been developed, the Oasis system is considered significant in the development of recycling systems for long duration space flight.

Energy largesse

The Administration's budget, which will be unveiled later this month, will contain some $1,570 million for energy research and development in the United States, according to Roy L. Ash, Director of the Office of Management and Budget. In an interview with a wire service reporter, Ash said that the Administration has accepted the spending proposals for the 1974–75 financial year contained in the energy research and development plan drawn up last month by the Chairman of the Atomic Energy Commission. The energy research budget next year will thus be some $700 million above the budget for this year. It will be interesting to see where the money comes from.

news and views

Geomagnetism and climatic change

THERE is a remarkable similarity between the pattern of the rth's magnetic field over the northern hemisphere, as disayed by the isopleths of magnetic intensity, and the mean ight contours of the surfaces of constant atmospheric pressure which define the shape of the circumpolar vortex of (broadly) westerly upper winds which prevail through most of the depth of the troposphere and lower stratosphere. Both patterns have an elongated, dumb-bell-like or bipolar form, at least when averages over a number of years are considered. In both cases, one end of the long axis is in the general region of the Canadian Arctic Archipelago, where the magnetic pole lies, and the other end is over northeast Siberia. In spite of its striking nature, this near-parallelism has received no more than passing notice in the literature of science. In an article on page 131 of this issue of *Nature*, King, of the Appleton Laboratory, Slough directs attention to the matter and adduces a number of other associations between the Earth's magnetic field and the atmospheric circulation and climate. These associations call for serious discussion and investigation, as they may indicate that the parallelism has a physical explanation, possibly of predictive value, in connection with long-term trends in climate.

Among the additional associations claimed by King perhaps the most impressive is just that the distinctive form of the isopleths of constant magnetic intensity over the southern hemisphere, much more nearly circular than in the north but eccentric in relation to the geographical pole with the pattern centred in the Indian Ocean sector, again broadly parallels the prevailing flow of the atmosphere (which has only been established by observation in recent years, roughly since the International Geophysical Year).

Meteorologists have been shy—perhaps understandably shy—about suggestions of a linkage, let alone control (as mentioned by King) of the flow of the atmosphere by the magnetic field, that is, ultimately by the flow in the Earth's molten core. But both phenomena could be registering parallel responses to some other aspect of the physical environment. The reluctance of meteorologists to consider these things doubtless has something to do with the fact that the atmosphere's dynamics and thermodynamics have long been explained adequately for most purposes (but perhaps not for the understanding of all long-term changes affecting climate) in terms of the major items of the energy budget at the Earth's surface and within the atmosphere, together with the effects of friction and distortion of the atmospheric flow by the greatest mountain barriers. Recent years have seen an impressive development of skill in forward calculation of the state of the atmosphere, based on these premises, using the capacity of the giant computers in the service of numerical methods of weather forecasting over one to five days ahead. It is natural that those who have been most closely involved in that particular advance of science should be a little reluctant to admit the need for other considerations in connection with long-term climatic development and changes—for example, the slow cumulative effects of extraneous influences, terrestrial and extra-terrestrial, and their possible trigger effects—and, perhaps, the appropriateness of quite other methods. It is far from clear, however, that the models used in numerical daily weather forecasting or any climatic models so far in sight will bring any advance at all in the capacity of scientists to forecast the weather a month or more ahead or to meet the demand for prediction of the climatic trend over years and decades ahead; though models can throw light on probable side effects of human activity (for example, increasing the carbon dioxide content of the atmosphere) which may modify the climate of the Earth.

King presents some further riddles in his article, in which a parallelism appears between the long-term displacement of the non-dipole element of the Earth's magnetic field and the behaviour of the atmospheric circulation over the northern hemisphere and its climatic effects suggest parallel histories over recent decades and centuries. Several of these apparent associations may turn out to be of only passing duration, and hence illusory, or they may be no better than loose and qualitative; but the facts will never be known unless they are investigated with the fullest assembly of long-term data that can be marshalled to the task. There is a clear, and urgent, need to identify and understand the physical processes that are involved in climatic changes—a need stressed, even before the current energy crisis, by the upsets to the international economy during the past 5 to 15 years by the long-continued, and increasing, drought in parts of Africa and several individual years of harvest failure over wide areas in India, China and the Soviet grainlands in central Asia.

Among the variables that have to be brought into the consideration of how climate varies, solar disturbance and minute, but directed, inputs of energy in high geomagnetic latitudes are often suggested. The ionosphere is well known to be affected by solar activity. Any responses in the lower atmosphere or, through the agency of geomagnetic disturbances, in the Earth's molten core are likely to differ with the time-scale of the external trigger operation; but they may show some common features and they are perhaps both likely to be more important in the case of long-lasting changes, however small in origin. One noticeable difference may already have been observed, which should warn meteorologists against expecting too close, or too complete, a paralellism: there is no suggestion so far that the geomagnetic field has registered a three-pole field over the northern hemisphere like that which has often appeared in the northern hemisphere atmosphere during the past 13 years (the third pole being near Novaya Zemlya.) Surely, however, no such strong hints of association as King points to should be left uninvestigated, especially when better knowledge of the processes of climate and climatic change is urgently needed. Another possibility is that such investigations may throw some light on the probable evolution of the flow in the Earth's molten core.

H. H. L.

Search for catecholamine receptors

Considerable progress has been made recently in the identification of membrane-bound receptor structures for polypeptide hormones such as insulin, glucagon and corticotrophin in mammalian tissues and cholinergic receptors in the electric organs of fish and eels. These studies have been based on the use of radioactive hormones or antagonists which bind very selectively to the receptor sites. Such binding if it is to be a valid method for identifying the receptors should show a very strict specificity, it should be saturable and indicate a finite and usually small number of receptor sites, and the affinity and rate constants for the binding reaction should correlate with the known biological properties of the receptor stimulant or antagonist molecule used. These criteria have been fulfilled in the instances just cited, and similar approaches have been successfully used to identify cholinergic muscarinic binding sites in mammalian brain, glycine receptor sites in spinal cord and receptors for morphine and other opiate drugs in brain (Hiley *et al., Biochem. J.,* **127,** 86P; 1972; Young and Snyder, *Proc. natn. Acad. Sci. U.S.A.,* **70,** 2832; 1973; Pert and Snyder, *Science, N.Y.,* **179,** 1011; 1973).

Cuatrecasas and colleagues, however, in last week's issue of *Nature,* (**247,** 92; 1974), illustrate the many pitfalls that await the unwary in using this apparently simple experimental approach for identifying receptor structures. Tritium-labelled noradrenaline of high specific activity has been available for some years, and might seem to offer a simple approach for labelling and identifying catecholamine receptors in mammalian tissues. Indeed Lefkowitz and his colleagues (*Proc. natn. Acad. Sci. U.S.A.,* **68,** 1773; 1971; *J. biol. Chem.,* **248,** 342; 1973) have claimed that the binding of ^{3}H-noradrenaline to fragments of cell membrane in microsomal fractions from mammalian heart and liver represents a specific binding to β adrenoreceptors in these tissues. Cuatrecasas *et al.* have carefully assessed this claim and their article clearly refutes it.

The binding of labelled noradrenaline to membrane fragments from liver, heart and fat cells or to intact fat cells was saturable, but the number of binding sites present in fat cells was three to four orders of magnitude greater than the number of insulin or glucagon receptor sites measured previously in these cells. Furthermore, the binding of ^{3}H-noradrenaline did not obey the strict specificity that would be predicted if this binding did represent labelling of β adrenoreceptors. For example, the non-radioactive (+) and (−) stereoisomers of noradrenaline had identical affinities as competitive antagonists of ^{3}H-noradrenaline binding. The affinity constant for such inhibition was more than 1μM, whereas the apparent affinity constant of (−) noradrenaline at β adrenoreceptors in fat cells was less than 0.1 μM, and (+) noradrenaline was at least one thousand times less potent in eliciting β-adrenoreceptor responses. ^{3}H-noradrenaline binding was also antagonised quite effectively by various catechol compounds, including pyrogallol and catechol acids, none of which have any activity at all as β-adrenoceptor stimulants, nor do they antagonise the receptor actions of (−) noradrenaline or isoprenaline. On the other hand, neither the non-catechol compound soterenol, which is a potent stimulant of β adrenoreceptors, nor the β-adrenoreceptor antagonist drug propranolol had any marked inhibitory effects on the binding of ^{3}H-noradrenaline.

It is thus clear that the structure responsible for the membrane binding of ^{3}H-noradrenaline is not identical with the β receptor for catecholamines. Indeed the specificity of these binding sites for catechols and their sensitivity to known inhibitors of the enzyme catechol-0-methyl transferase (pyrogallol, tropolone, quercetin) lead Cuatrecasas *et al.* to conclude that these binding sites more probably represent a membrane-bound form of this enzyme, known to exist in the microsomal fractions of the tissues studied.

The use of labelled hormones and neurotransmitters or their antagonists is likely to remain a most fruitful approach to identifying elusive membrane-bound receptor sites. But since such receptors are present in most tissues in extremely small numbers such potential labels must bind with extremely high affinity, must be available with extremely high specific activity and must obey very precise specificity rules in order for this approach to be valid. The hunting of the receptor snark can otherwise all to easily lead to boojums.

From our Neuropharmacology
Correspondent

Selective adhesion and retinotectal specificity

from a Correspondent

The development of the brain involves the segregation of vast numbers of cells into precisely delineated subpopulations which are linked by highly selective neuronal connections. The mechanisms responsible for the formation of specific nerve connections have been analysed, most extensively in the retinotectal system of lower vertebrates: the axons of retinal ganglion cells connect selectively at local tectal sites to produce a map of the retina across the surface of the optic tectum. The most popular hypothesis that seeks to account for the discrimination in intercellular recognit[illegible] that such selective connections impl[illegible] that due to Roger Sperry, who s[illegible]gested, some 30 years ago, that cellular differentiation in the nervous system extends beyond the level of neuronal populations to that of the individual neurones themselves. The differentiated neurone acquires a characteristic cell surface label which participates in selective intercellular recognition and the formation of synaptic connections.

This hypothesis of neuronal specificity has withstood the test of time with remarkable success. Recent work has concentrated not so much on the hypothesis itself as on the rules by which such labelled neuronal arrays interconnect. The experimental paradigm adopted to examine the rules involves the surgical creation of relative size disparities between the innervating cell population and its target. Thus the patterns of connection that result when half a retina is caused to innervate a whole optic tectum or, conversely, when a whole retina feeds into a tectum half of which has been removed, have been described. The interpretation of such results has, however, run into a conceptual problem. One does not know whether the altered pattern of connections that is frequently found reflects altered neuronal labels, occurring as the direct result of experimental interference with a neuronal population or whether, alternatively, the neuronal labels themselves are unchanged by the operation. In this second case, the altered connection pattern might indicate the presence of a competitive mechanism in the formation of synaptic connections which are, then, determined not solely by the 'specificity labels' the[illegible] neurones carry but also by the context in which the two extant neuronal populations are interconnecting (Gaze and Keating, *Nature,* **237,** 375; 1972). The inability to distinguish between these two alternatives will persist until the appearance of an experimental design in which it is possible to deduce the nature of the neuronal labels independently of the formation of synaptic connections by the neurones.

One must await either the identifica[illegible]n of the 'specificity molecules' or, alternatively, an assay system which reveals their presence by a criterion other than the formation of synapses. What might be the first, and very exciting, step in the direction of such an assay is provided by the very elegant experiments of Barbera, Marchase and Roth (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2482; 1973).

Building upon earlier work which had demonstrated differences in adhesability between cells from different tissues, and from different brain regions, Barbera, Marchase and Roth decided to investigate whether there is preferential adhesion between cells which will become [illegible]ronally linked as opposed to cells that [illegible] not neuronally link. In their assay system a ^{32}P-labelled cell suspension of either dorsal or ventral retina of embryonic chicks was placed in dishes containing both ventral and dorsal tectal halves. Cells from the dorsal retina were found to adhere preferentially to ventral tectum and cells from ventral retina adhered preferentially to dorsal tectum. This preferential adhesion is similar to the innervation pattern of the retinotectal system; dorsal retina normally connects with ventral tectum and ventral retina with dorsal tectum.

This interesting result is interpreted with appropriate caution by the authors. Indeed, some care is warranted fore it can be accepted that the cell face recognition phenomenon being onstrated here is the one involved in specific synaptogenesis. Only a small proportion of the cells demonstrated the preferential adhesion that the authors monitored; most of the cells were easily displaced by light washing. Of those cells that did display the preferential adhesion, the difference in number between those adhering to the 'correct' tectal half exceeded those in contact with the 'wrong' half only by a factor of two, which is rather low for a system in which the differential specificity of neuronal connections is, in most models, viewed as very high.

As to the question of whether the mechanisms involved in cell adhesion are those involved in synapse formation, it must be accepted that the greatest part of the work on selective cell adhesion has emphasised the tissue-specific nature of these adhesions. Thus retinal cells selectively adhere to retinal cells, cerebellar cells to cerebellar cels (see Luis and Glaser (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2794; 1973)). This tissue-specific aggregation probably involves quite different surface receptors from those involved in synaptogenesis, because most neuronal connections link different populations rather than cells of the same population. In this context it is interesting to note that retinal pigment cells display the same preferential adhesion to tectal halves as do the neural retinal cells, even though retinal pigment cells are not neurones and most certainly do not form synaptic connections with the tectum.

Despite these caveats, this work of Barbera *et al.* is a most promising advance in attempts to understand the mechanisms of selective cell recognition. Such mechanisms will throw light on the formation of tissues and these experiments suggest that similar processes may underlie the elaboration of the intricate pattern of synaptic linkages that characterises the functioning nervous system.

Endemism and environmental stability

from our Plant Ecology Correspondent

ENDEMIC organisms and the problems underlying their origin and survival have a peculiar fascination for biogeographers. As long ago as 1882 Engler had postulated that there are basically two types of endemics, ancient ones (palaeoendemics) and those of recent origin (neoendemics). Various criteria have been used for their differentiation, the most effective being the geographic proximity of closely related species. Palaeoendemics are normally disjunct in distribution, occurring in situations well isolated from similar species. Neoendemics, on the other hand, often have many closely related species in the same or adjacent areas.

Stebbins and Major (*Ecol. Mongr.*, **35**, 1; 1965) analysed the flora of California, where roughly a third of the indigenous plants are endemic, and concluded that the palaeoendemic species are chiefly found in either markedly damp or distinctly dry environments, whereas intermediate climatic regimes are typified by neoendemics. They explain this by suggesting that palaeoendemics are found only in stable climatic situations whereas an unstable climate is conducive to rapid extinction and evolution, resulting in the production of neoendemics.

Ultimately, the demonstration that long-term climatic stability is associated with palaeoendemism is in the hands of the palaeoecologist. Meyer (*Ecology*, **54**, 982; 1973) has now accomplished this in the Cuatro Ciénegas Basin in northern Mexico, another area rich in endemics. The Cuatro Ciénegas Basin lies at an altitude of 740 m and is surrounded by mountains rising to heights in excess of 2,500 m. Many aquatic animals are endemic in the basin, including nine species of fish, three isopod genera and thirteen species of hydrobiid snails. The character of this fauna suggests that long-term isolation, perhaps accompanied by climatic stability, has resulted in its survival and development. In other words, these organisms are palaeoendemics.

Meyer has analysed palynologically two cores from the central area of the basin in order to reconstruct the environmental history of the region. One of these cores, which was taken from a spring-head mire, reaches a depth of 13 m, and radiocarbon dating on material between 11 m and 13 m gave results of >31,400 BP and >26,800 BP. The record thus goes back to the middle of the last (Wisconsin) glacial advance, at which time the area would have been experiencing pluvial conditions.

The pollen diagrams are remarkably uniform and give no evidence of rapid climatic fluctuations. The main fluctuations are in the pollen of grasses and the Chenopodiaceae / Amaranthaceae group, which probably reflect local changes in soil salinity and hydrology rather than climatic change. Modern pollen grain studies in the region indicate that tree pollen (mainly *Pinus*, *Quercus* and Cupressaceae) is derived largely from the montane *Pinus ponderosa* forests and Compositae pollen comes mainly from the mixed xerophytic communities and creosote bush savannah of the basin floor. Since the tree pollen:Compositae ratio remains fairly constant throughout the profile, it is reasonable to postulate a long term stability in these major vegetation types.

In this particular locality it is therefore possible to associate a high incidence of palaeoendemism with a history of environmental stability, thus providing support for Stebbins and Major's hypothesis.

Some somatic cell variants are mutants

from a Correspondent

ARE somatic cell variants true mutants, that is, are they really the result of stable and hereditary changes in nucleotide sequence? This question, which surely has disturbed the sleep of many a somatic cell geneticist, receives two positive replies in the November issue of the *Proceedings of the National Academy of Sciences*.

First, Thompson, Harkins and Stanners (**70**, 3094), continuing the Toronto group's work on temperature-sensitive (*ts*) variants, report that one of their *ts* Chinese hamster cells, *ts*H1, has a *ts* leucyl-transfer RNA synthetase. Mutant *ts*H1 grows normally at 34° C, but after shift up to 39.5° C, cell division stops and the cells disintegrate within 24 h. The mutant shows no subclone variation, has a low reversion frequency which is increased by exposure to mutagens such as ethyl methanesulphonate, and is recessive in cell hybrids. The clue that *ts*H1 has a primary defect in protein synthesis came when the rates of DNA, RNA and protein synthesis were examined after cells were placed at the nonpermissive temperature. Both DNA and RNA synthesis decline with a half-life of between 5–8 h; notably, protein synthesis shuts off almost immediately and is down to 5% of control values by 1 h.

Following this lead, Thompson *et al.* looked for various amino-acylated tRNAs by feeding cells ^{14}C-amino acids in the presence of cycloheximide and actinomycin D. Wild-type cells and a temperature-resistant revertant showed equal amounts of leucyl-tRNA at both 34° C and 40.5° C; *ts*H1 had normal amounts of all the amino acyl-tRNAs at 40.5° C, except leucyl-tRNA which was reduced more than 100 times. Direct assay *in vitro* of the leucyl-tRNA synthetase from *ts*H1 showed that it is the synthetase itself which is *ts*.

The second report, by Sharp, Capecchi, and Capecchi (**70**, 3145), is a study of the properties of the favourite enzyme of the somatic cell geneticist—hypoxanthine phosphoribosyl transferase (HPRT)—in variants of the mouse L cell. Cells that have a normal HPRT are extremely sensitive to the purine analogues, 8-azaguanine (8AG) and 6-thioguanine; revertants can be isolated using the Littlefield technique of blocking *de novo* purine biosynthesis with aminopterin and simultaneously providing hypoxanthine and thymidine ('HAT' selection). While looking for nonsense suppressors, Sharp *et al.* examined the kinetic properties and heat stability of HPRT among such HAT-resistant revertants. Several revertants grew equally well in both medium used to isolate HPRT-negative cells (8AG) and in medium used to isolate HPRT-positive revertants (HAT). These revertants had high concentrations of HPRT.

Initially, this seems to be a surprising, if not a slightly disturbing, result, though it would be expected if the revertant enzyme had a decreased affinity to 8AG, but had the normal K_m for hypoxanthine. This is precisely what Sharp *et al.* report for one such revertant, suggesting that a structural change has occurred in the enzyme. Another class of revertants possessed a very heat-labile HPRT, again providing strong evidence of structural changes in the enzyme as the result of mutation.

These two reports, taken together with previous findings of 8AG-resistant variants that contain material that cross-reacts immunologically with HPRT but have no enzymatic activity (Beaudet *et al.*, *Proc. natn. Acad. Sci., U.S.A.*, **70**, 320) and α-amanitin-resistant mutants due to an altered RNA polymerase II (Chan *et al.*, *ibid.*, **69**, 3119), should help to quiet the critics. It seems that at least some variants are mutants.

Analytical techniques for museums

from a Correspondent

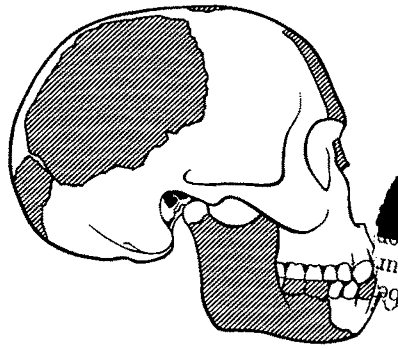
THE Society for Analytical Chemistry (Midlands Region) recently cooperated with the United Kingdom Group of the International Institute for Conservation in organising a symposium at the Birmingham Museum and Art Gallery on December 4. The scope of the contributions concerned both the present types of chemical analytical methods used in museum laboratories and also reviews of techniques which, although used increasingly in chemistry research in general, may also have applications to the examination of museum objects.

B. W. Fitzsimmons (Birkbeck College, London) described the theory and practice of Mössbauer spectroscopy and its applications to the study of ^{57}Fe and ^{117}Sn nuclei. As this technique gives information on the chemical environment of iron and tin, its potential use in museums is in the examination of clays and iron oxide pigments which may be complex mixtures of ferrous and ferric compounds. M. Thompson (University of Technology, Loughborough) dealt with photoelectron spectroscopy. Using X-ray excitation all elements except hydrogen can be detected in small samples, and it is possible to deal with many mixed oxide systems. The extreme sensitivity and high selectivity of the antigen-antibody reactions of, for example, proteins in immunological methods (J. N. Miller, University of Technology, Loughborough) which are already used in forensic science and food science suggest that they could be applied to paintings and other art work to reveal the media used by the artist. A key issue is whether antigen activity survives over long periods and this requires investigation.

After descriptions of potential new techniques, the methods used at present and results obtained in different museum laboratories were then described. J. Mills (National Gallery, London) has used infrared spectrometry and chromatography, principally GLC, to identify organic materials in art objects. Organic materials encountered in antiquities, especially paintings, are often complex natural products, but GLC of terpenoids (that is, resins) has provided new information on their identification and the chemical changes undergone over long periods. Ancient bronzes and faience in numbers running into the thousands have been analysed by H. McKerrell (National Museum of Antiquities of Scotland) using the version of non-dispersive X-ray fluorescence which he has developed. A rapid non-destructive method such as this has gre[illegible] potential for analytical surveys of large groups of museum objects. Such analyses have an important bearing on basic archaeological problems of trade contacts and sources of raw materials such as tin. In another laboratory (M. J. Hughes, Research Laboratory, British Museum) a combination of qualitative spectrographic and quantitative atomic absorption analysis has been used, because these methods are sufficiently sensitive for analyses of the small samples only which can be taken from museum objects.

Where expensive analytical equipment is not available, J. Hedley and A. Cumming (Courtauld Institute of A[illegible] London) described how opti[illegible] mineralogy, microchemical analysis and spot tests can be applied to inorganic materials used in paintings, and for organic compounds solubility tests and selective staining have been successfully applied.

Piltdown Man



THE Piltdown Man hoax still arouses curiosity and indeed was the subject of a recent British television programme some 60 years after the bits of skull and jawbone were found in a remote corner of Sussex. The British Museum (Natural History) has therefore done a service to the ordinary person who likes a scientific detective story, by producing a slim guide (*The Piltdown Man Hoax*, Palaeontology Leaflet No. 2, pp. 6; 7p) which sets out simply how the Piltdown remains came to be found, names the persons chiefly responsible for identifying the fragments, describes how the hoax was exposed at the museum and elsewhere between 1949 and 1953 and explains how, in 1973, the museum can say confidently "none of the *Eoanthropus* fragments can be considered fossil or in any way part of man's ancestry".

Weather and the Earth's magnetic field

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A comparison of meteorological pressures and the strength of the geomagnetic field suggests a possible controlling influence of the field on the longitudinal variation of the average pressure in the troposphere at high latitudes. If so, changes which occur in the pattern of 'permanent' depressions in the troposphere as the magnetic field varies (for example, as the non-dipole component of the field drifts westwards) may be accompanied by climatic changes.

I HAVE recently suggested[1] that regions of low atmospheric pressure observed near the magnetic poles occur because these regions, which are linked magnetically to the interior of the magnetotail, are strongly shielded against the incursion of olar particles. Although the mechanisms by which energetic particles may affect the troposphere are unknown, in this paper I describe the possible influence of the Earth's magnetic field on the height of the 500 mbar level in the regions surrounding the northern and southern magnetic poles. It will also be shown that certain features of the 'permanent' atmospheric pressure system seen to have moved westwards during some decades of the present century; this movement may be associated with a corresponding westward drift of the magnetic field pattern.

Figure 1 shows the average height of the 500 mbar level for January in the northern hemisphere[2]; the contours exhibit the typical northern hemisphere winter pattern of two lows, one near 80°W and the other near 130°E. Other examples of average pressure maps for the northern hemisphere have been published[3] and they all show a highly elongated or 'dumb-bell-shaped' low at high latitudes.

Comparison with magnetic parameters

Various theories[2] have been advanced in order to explain the existence and location of the two lows observed in the northern hemisphere, but theoreticians working in this field do not seem to have taken into account the fact, pointed out by other authors[4-9] in the past, that the Earth's magnetic field and the behaviour of the lower atmosphere may be connected in some way.

Figure 2 contains a map[10] showing contours of constant magnetic intensity, B, of the Earth's magnetic field (for middle and higher latitudes) at an altitude of 400 km in 1965; the northern hemisphere data are replotted in the polar plot shown in Fig. 3. The height for which the magnetic data are presented was chosen arbitrarily; the contours for lower altitudes would not have significantly different shapes, merely different B values.

A comparison of the longitudinal variations, at 60°N, of the 500 mbar data from Fig. 1 and the magnetic B data from Fig. 2 is shown in Fig. 4. The similarity between the two curves is striking except that the magnetic-B curve is displaced about 25° towards the west. Although the meteorological data relate to an earlier epoch (1918–1958) than the magnetic data (1965), no attempt has been made to correct the magnetic data for the well-known westward drift[11,12] of the non-dipole component of the Earth's magnetic field; part of the phase difference between the two curves would be removed by taking this into account. (A comparison of magnetic dip or magnetic declination maps from different epochs of the present century shows that the magnetic field patterns are rotating westward on average about 3° per decade; the drift is by no means uniform over the Earth's surface and it varies from decade to decade). The remaining phase difference may arise because of a complicated phase relationship between the atmospheric response and the modulating force imposed by the unknown magnetic-field-dependent 'driving force.'

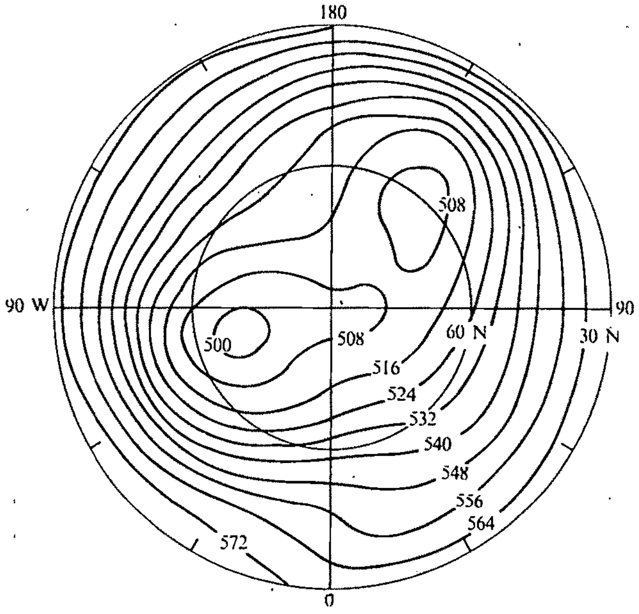

FIG. 1 Northern hemisphere polar plot showing the average height (decametres, dam) of the 500 mbar level for January. (After Palmen and Newton[2]).

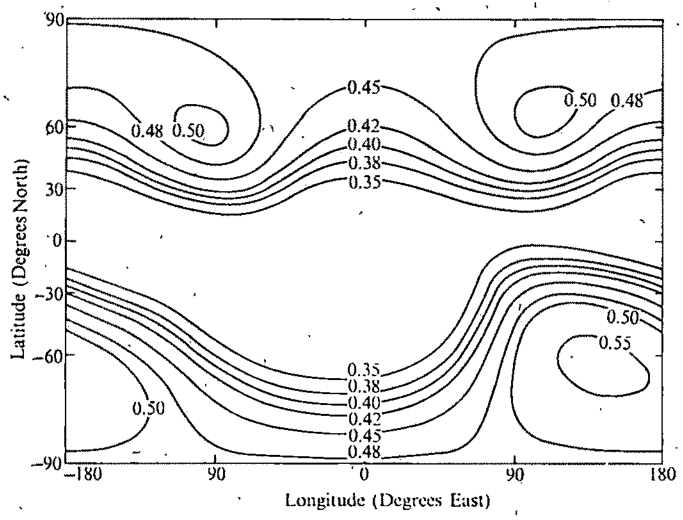

FIG. 2 Map showing contours of constant magnetic field strength B calculated for 400 km, 1965. (After Stassinopoulos[10]).

It should be noted from Fig. 4 that the magnitude of the 500 mbar height change associated with the magnetic field is of the order of 250 m. Comparison of the meteorological data (Fig. 1) and the magnetic data (Fig. 2) reveals that the pressure lows are centred on latitudes of 70° and 62° whereas the magnetic-B maxima occur at latitudes of 61° and 66° respectively.

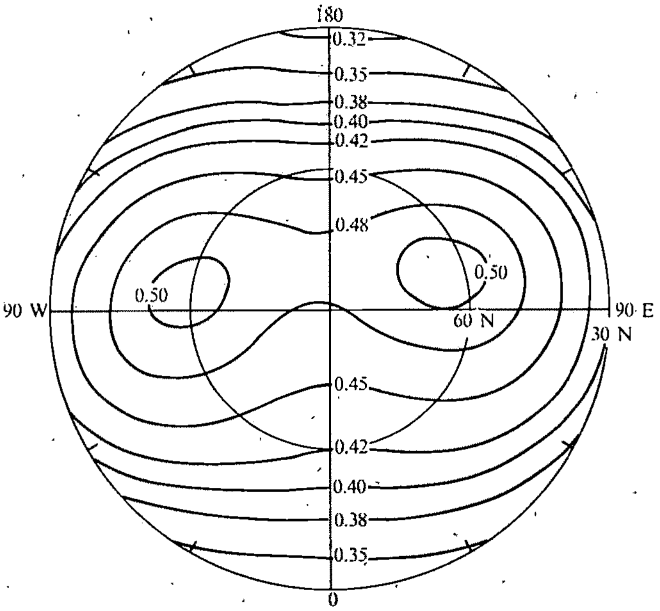

FIG. 3 Northern hemisphere polar plot showing contours, drawn from Fig. 2, of constant magnetic field strength for 400 km, 1965.

Figure 5 contains maps showing the height of the 500 mbar winter isobars over most of the northern hemisphere for epochs centred on 1933 and 1951 respectively. (The older data were obtained from ref. 13 and related to the December–February season. These data were compiled from observations reported in thirty-three documents, ten of which indicated in their titles the years for which the data applied; the average of these years was 1933. The other twenty-three documents had an average publication date of 1938 and it appears reasonable to conclude that the results are representative of years around 1933. The more recent data[14] related to January during the years 1949–53).

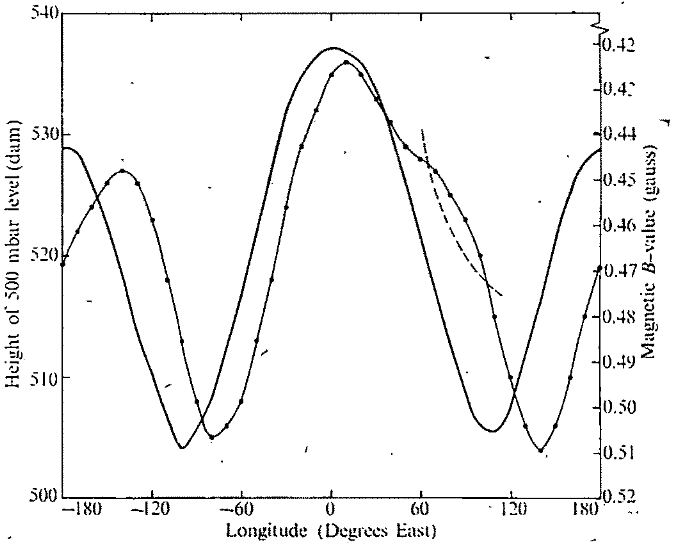

FIG. 4 Curves showing: •—•, the longitudinal variations at 60°N of the height of the 500 mbar level (from Fig. 1); —, the magnetic field strength (from Fig. 2). The short broken curve draws attention to some of the pressure data which may be anomalously high. The magnetic data relate to 1965 and the meteorological data to the epoch 1918-58.

Although statistically stronger evidence would be desirable, Fig. 5 does indicate that the average high-latitude pressure distribution appears to have moved westward by about 12° between 1933 and 1951; this angle is greater than that through which the Earth's magnetic field pattern rotated during the same period. Further information about the apparent westward rotation of the average pressure system is shown in Fig. 6 which relates to 60°N over the western hemisphere. Examination of the longitudes at which the maxima and minima occur shows that, averaging over summer and winter and heights between 300 and 700 mbar, the average pressure system moved about 15° westward between 1933 and 1951. It should be pointed out that the

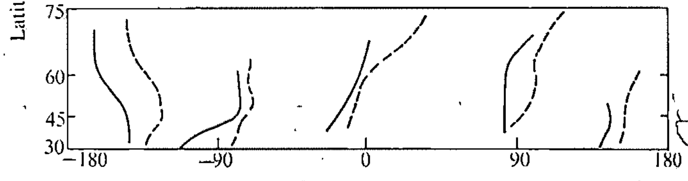

FIG. 5 Maps of the height of the 500 mbar isobars in winter. Upper: December-February, for an epoch centred on about 1933. The contours range from 500 to 567 in eleven steps of 6.1 dam. (After ref. 13). Middle: January, for the five-year period centred on 1951. The contours range from 500 to 570 in seven steps of 10 dam. (After ref. 14). Lower: Curves showing the positions of the crests and troughs appearing in the upper two sections. - - -, 1933; —— 1951.

recent data[14] related only to January or July whereas the older data[13] are for the three-month periods December–February or June–August; although significant differences exist between the average pressure distributions for different seasons, examination of all the maps available to the present author indicates that the seasonal variations cannot vitiate the conclusion that some parts of the average pressure system appear to be drifting slowly westward.

In the southern hemisphere the magnetic invariant pole is situated at a longitude of 125°E. It is well known that the geomagnetic contours surrounding the south magnetic pole are much more circular than the highly elongated ones around the northern pole and, therefore, the magnetic field should not be expected to produce meteorological effects at two longitudes in the southern hemisphere. Lamb (ref. 3, page 29) has published a polar diagram showing the mean height of the 500 mbar surface in the southern hemisphere; the low at the centre of the system is slightly displaced from the geographic pole, along the 120°E meridian. Figure 7 has been obtained from Lamb's plot and shows the latitudinal variation of the pressure down the 120°E meridian, across the geographic pole and up the 60°W meridian. The effect of the assumed magnetic-field-dependent mechanism seems to be a reduction in the height of the 500 mbar surface by a maximum of 140 m at 60°S. Figure 2 shows that the highest B-values in the southern hemisphere at 120°E also occur at 60°S; the height of the 500 mbar level is thus most reduced at the latitude where the magnetic field is strongest.

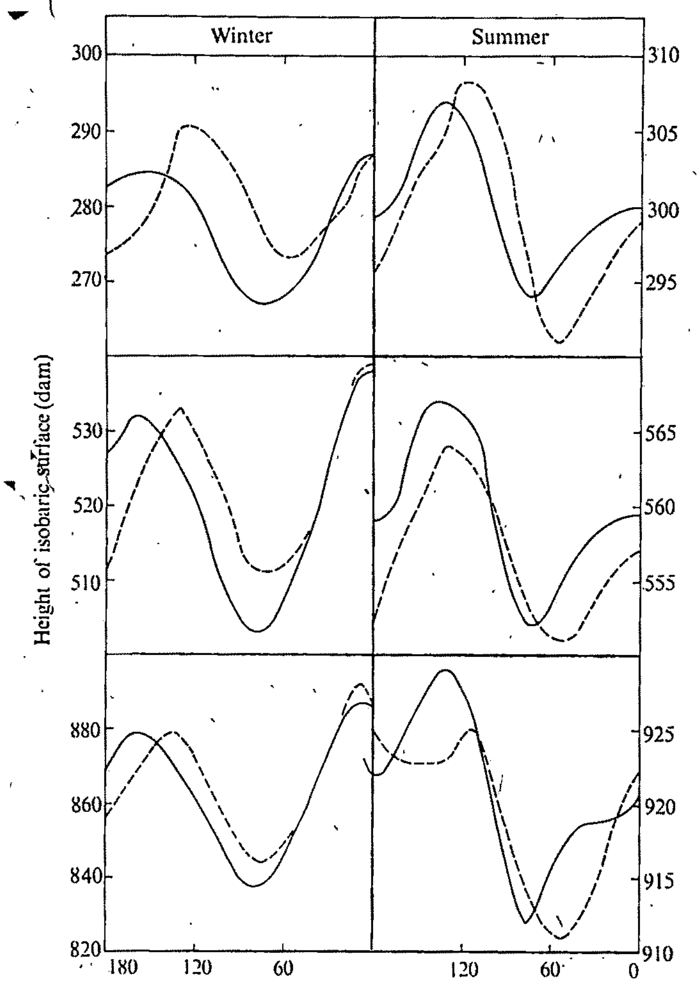

Fig. 6 Longitudinal variations of the height (dam) of the 700 (top), 500 (middle) and 300 (bottom) mbar levels in winter and summer for two epochs. – – – –, 1933; ——, 1951. (refs 13, 14).

Implications and applications

I conclude that: (a) the Earth's magnetic field possibly influences the average tropospheric pressure system at high latitudes; and (b) the average pressure system seems to move westwards as the magnetic field rotates. The evidence relating to the second point is less convincing and there is clearly a need for further comparisons of meteorological data from widely spaced epochs for which reliable maps of magnetic field strength are also available. Even though Fig. 5 may not provide conclusive evidence about the westward motion of the pressure system, it does show that the pressure contours are very similar to the magnetic contours drawn in Fig. 2.

The large geographical extent of the permanent depressions results in considerable meridional air motion; warm air from lower latitudes at the longitude of a depression will move towards higher latitudes to the east of the depression. Clearly, if the longitude of one of the northern hemisphere depressions drifts towards the west the climate over much of the northern high-latitude region will alter. It should be noted, in this context, that the magnetic declination at London and Paris was zero in about 1660 (refs 15, 16); the magnetic field configuration then prevailing in the northern hemisphere was obviously very different from the present one and it is interesting to note, therefore, that Europe and certain other parts of the northern hemisphere experienced a "Little Ice Age"[3] during the period 1550–1700 AD. It would be expected, if the average tropospheric pressure systems are indeed controlled by changes of the Earth's magnetic field, that various climatic phenomena which have occurred in the past should be associated with changes in the magnetic field. Evidence that this is so will be presented elsewhere.

The phase difference between the longitudinal pressure and magnetic variations (Fig. 4) can only be partially explained by the westward drift of the Earth's field. I suggest that the remaining difference (between the phase of an unknown magnetic-field-dependent driving force and the response of the atmosphere) may be caused by Coriolis force

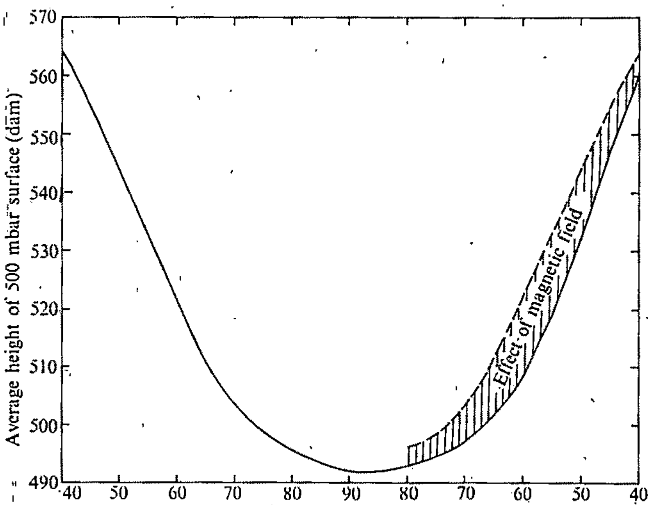

Fig. 7 Latitudinal variation of the height of the 500 mbar surface down the 120°E meridian and up to 60°W meridian. The difference between the values along the two meridians is greatest near 60°S. —— Observed; – – –, mirror image of data from 60°W meridian.

and by the inertia and viscosity of the air. If Coriolis force is important in this connection, the phase difference should be expected to vary with latitude, probably becoming greater at lower latitudes. The phase difference will also, of course, depend on the process by which energy is imparted to the atmosphere and thus the observed phase differences, when the effects of Coriolis force and so on have been subtracted, may constitute an important clue in the search for trigger mechanisms that enable changes in the solar wind or other forms of solar radiation to affect the vast mass of the lower atmosphere.

The meteorological data in Fig. 4 exhibit some irregular behaviour which is most pronounced in the longitude range 70 to 100°E, exactly the range covered by the highest mountains of Central Asia. If the longitudinal variation of the average height of the 500 mbar level at 60°N is dependent on the Earth's magnetic field, it may not be necessary to invoke orographic (related to mountains) or thermal effects associated with topographical phenomena such as land/sea boundaries to explain the major features observed. Possibly the main effect of mountains on the average height of the 500 mbar level at 60°N is the small increase, approximately 30 m, observed at the longitude of the mountains of Central Asia.

The conclusion that some unknown magnetic-field-dependent mechanism seems to modulate the tropospheric pressures by an amount which is greater in the north than in the south is particularly interesting because it is known[17] that the intensity of auroral optical emissions, produced by energetic particles impinging on the atmosphere, is 30% greater in the northern than in the southern hemisphere.

It has recently been demonstrated that changes in the sector structure[18] of the interplanetary magnetic field, and also changes of geomagnetic activity[19,20], are correlated with the depths of meteorological troughs crossing North America. Other workers[21] have also reported that conditions in the troposphere appear to be abnormal near the times of geomagnetic disturbances. The explanation of these results may be similar to that of the marked influence which the Earth's magnetic field appears to exert on the troposphere. Some of the studies[22] involving correlations between the troposphere and the sector structure have suggested why solar-weather relationships established empirically for certain periods are not observed during other epochs.

The problem of the trigger mechanism by which small amounts of energy from the solar wind can affect the atmosphere provides an interesting field of research. Roberts and Olson[19] have suggested that cirrus cloud formation following ionisation produced by X-rays emitted during aurorae may be important; a difficulty about this suggestion is that auroral bremsstrahlung do not normally penetrate to tropospheric heights. Energetic solar protons and γ rays associated with heavy particles are, however, capable of reaching lower heights than the bremsstrahlung and it may be that they affect the atmospheric pressure in some way. It is known, alternatively, that changes in the solar radiation affect the stratospheric ozone concentration[23,24] and perhaps, therefore, the thermal balance of the lower atmosphere.

It seems possible that the solar wind may influence the behaviour of the Earth's atmosphere if the accretion of solar wind protons results in the production of water vapour in the stratosphere[25,26]. De Turville[25] suggested that all the water in the oceans might have been formed in this way, but concluded later[27] that this was not the case; the considerations which led to the withdrawal of the suggestion involved the rate at which water vapour would be produced rather than whether the process occurred at all. Wasson and Junge[28] concluded: "The importance which is attached to an extra-terrestrial orgin for even part of the stratospheric water-vapour means that water-vapour measurements, especially in the polar stratosphere, could be of great scientific value". It is well known that the penetration of the solar wind to the near-environment of the Earth depends on the terrestrial magnetic field and it would seem worth examining, therefore, whether the spatial distribution water vapour in the stratosphere, and hence the thermal balance of the lower atmosphere, is also dependent on the field. If the relatively low atmospheric pressures that occur in regions of high magnetic intensity are a consequence of shielding by the magnetic field against direct injection of certain solar wind particles it should be expected that, since the angle between the magnetic dipole and the solar wind is very different in summer from that in winter, solar-weather relationships will show marked seasonal changes. Roberts and Olson[20] have reviewed evidence which clearly shows that this is the case.

Although the evidence presented in this paper cannot be construed as proof that the Earth's magnetic field and the average tropospheric pressure patterns are related, it does suggest that more work on this problem should be undertaken.

I propose that the field of research involving inter-relatio ships between meteorological phenomena and processes d pending on the Earth's magnetic field should be known as 'magnetometeorology'.

I thank Drs W. C. Bain, H. H. Lamb, J. A. Saxton and D. M. Willis for comments and Dr D. L. Croom for drawing my attention to the stratospheric water vapour problem[25–28].

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Myosin from Cross-reinnervated Cat Muscles

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Correlated physiological changes, and structural and functional changes in myosin, following cross-reinnervation of cat fast-twitch and slow-twitch muscles, indicate that the myosin phenotype is controlled through the nervous system.

THE changes which occur in the isometrically recorded contractions of cross-reinnervated mammalian fast-twitch and slow-twitch muscles are now well documented[1–3]. In many respects, the study of isotonic shortenings can, however, be more directly informative about changes in the contractile machinery. Close, using the rat[2,4], first clearly demonstrated that cross-reinnervation of the soleus (SOL, slow-twitch) and extensor digitorum longus (EDL, fast-twitch) muscles produced reciprocal changes in both the maximum speed of shortening (V_0) and the curvature of the force-velocity relationship. More recently Bárány and Close[5], using the rat, ave shown that the activity of the Mg^{2+}-dependent actomyosin ATPase also changes following cross-reinnervation, in a manner which parallels the alteration in V_0. These findings supported Bárány's earlier hypothesis[6] that the actomyosin ATPase activity is the rate limiting step which determines a muscle's maximum shortening velocity.

Isotonic studies of cross-reinnervated cat muscles have given less clear cut results than in the rat. While cross-reinnervation of fast-twitch muscle by a nerve formerly innervating soleus (a slow-twitch) muscle produced a marked reduction in V_0, as in the rat, cross-reinnervation of the soleus muscle produced a variable but usually small increase in V_0 (ref. 7). Studies of the muscle myosin Ca^{2+}-dependent ATPase activity showed this to parallel the changes in shortening velocity[8].

A further study[9] of the isotonic shortenings of cross-reinnervated cat muscles using soleus as the slow-twitch muscle but the larger head of the deep digital flexors, correctly called flexor hallucis longus (FHL) rather than the formerly used smaller head (flexor digitorum longus, FDL)[7] as the fast-twitch muscle, has led to results which are in general agreement with those obtained in the rat. In particular, following cross-reinnervation, the soleus muscle showed a marked increase in V_0, a change which was accompanied by a decreased curvature of the force-velocity relationship. It was then of interest to know whether, as in former cases, the ATPase activities of the myosins from these muscles mirrored their shortening velocities, and further whether the myosin light chains were altered following cross-reinnervation. These low molecular weight proteins, which are part of the enzymatically active "head" of the myosin molecule, are chemically different in normal fast-twitch and slow-twitch muscles and their electrophoretic mobilities provide a characterisation of the myosin phenotype[10–12].

The results described in this paper were obtained from the muscles of four cats which had been cross-reinnervated for approximately 12 months and which formed part of a larger physiological study[9]. The four animals were selected because they showed a purity of reinnervation by the crossed nerve in excess of 90%. As the muscles were required for biochemical studies, histological examination was not undertaken, the success of the cross-innervation being gauged by the purity of the reinnervation and the fact that on indirect stimulation the cross-reinnervated muscles developed a mean of 91% of the maximum isometric tension of the control muscles. It was noted, however, that the cross-reinnervated soleus muscles tended to develop somewhat more tension than their controls and the cross-reinnervated FHL muscles less.

The apparatus and methods used have been described fully elsewhere[13]. Fibre lengths were measured by the method of Al-Amood and Pope[14]. At the end of the physiological experiments the muscles were excised, blotted and weighed, then frozen in liquid nitrogen and stored at −20° C.

Myosin was prepared essentially as described by Bárány and Close[5]. The muscles were chopped finely with a razor blade and connective tissue removed, then homogenised in 30 mM KCl, 5 mM potassium phosphate at *p*H 6.7. The myofibrils were pelleted by centrifugation at 10,000*g* and the supernatant used to measure the absorbance of the Soret band at 417 nm (for quantitation of haem proteins). The myofibrils were washed twice more with 20 volumes of the same buffer, then extracted in a medium containing 0.3 M KCl, 0.15 M potassium phosphate, 5 mM MgATP, 0.1 mM dithiothreitol at *p*H 6.6 for 20 min at 4° C with gentle stirring. The extract was centrifuged at 27,000*g* for 10 min and the supernatant dialysed overnight against 30 mM KCl to precipitate the myosin, which was collected by centrifugation at 10,000*g* for 15 min. The myosin precipitate was washed with 30 mM KCl to aid removal of excess nucleotides, dissolved at 0.6 M KCl, 20 mM potassium phosphate at *p*H 6.7 and clarified by centrifugation at 40,000*g* for 30 min. Myosin was reprecipitated by dialysis against 30 mM KCl, 2 mM potassium phosphate and finally dissolved in 0.6 M KCl, 20 mM potassium phosphate at *p*H 6.7 and clarified once again.

The myosin concentration was estimated by absorbance at 280 nm assuming as an extinction coefficient that obtained for rabbit fast muscle myosin ($E_{280}^{1\%}=5.60$ (ref. 15). The extinction coefficients measured for myosins prepared as described from frozen cat muscles were up to 25% higher than this value and SDS gels showed the presence of contaminating actin, which could not be completely removed without unacceptable loss of myosin. For this reason, in some preparations the extracted myofibrils were centrifuged at 165,000*g* for 4 h to pellet contaminating actin. The myosin was precipitated by dialysis against low salt, redissolved in 0.6 M KCl, 20 mM potassium phosphate at *p*H 6.7, and subjected to a second ATP dissociation followed by centrifugation at

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165,000g for a further 4 h to remove residual actin. Finally, the myosin was reprecipitated by dialysis, the precipitate washed to remove nucleotide and redissolved as above. These preparations, which were largely free of actin, gave extinction coefficients which were the same for both the SOL and FHL myosins ($E^{1\%}_{280}=6.2$, based on the Folin reaction using bovine serum albumin as standard). The need for accuracy in protein determination could be circumvented by the use of stopped-flow assays, which are independent of protein concentration. The molecular weight of myosin was taken as 470,000[16], and the molarity based on two equivalent structure units of 235,000 daltons. F-actin was prepared according to Kendrick-Jones *et al.*[17].

Solvent conditions for the different kinetic assays of the ATPase are described in Table 1 and Fig. 1. The steady state actin-activated Mg^{2+}-dependent ATPase was used most extensively. The Mg^{2+}-dependent ATPase was measured occasionally but required relatively large amounts of myosin. A further assay measured the rate of protein fluorescence increase when myosin was mixed with a large molar excess of ATP, using the stopped-flow fluorimeter technique of Bagshaw *et al.*[18]. With the experimental conditions used, the observed rate constant of this pseudo-first order reaction is directly proportional to the ATP concentration and independent of myosin concentration. This method has a second advantage that the observed rate is independent of small amounts of contaminating actin and thus two potential sources of error in comparing different myosins are eliminated. The percentage increase in fluorescence when ATP was mixed with fast-twitch and slow-twitch muscle myosins was taken to be the same, that is, 8±2% (Fig. 1).

Polyacrylamide gel electrophoresis in the presence of SDS was carried out according to Weber and Osborn[19]. The samples were mixed with a five-fold excess of SDS by weight and 1% β-mercaptoethanol and placed in a boiling water bath for 3 min. Aliquots of 50–150 μg were applied to 7 cm gels. Staining was in 0.2% Coomassie Brilliant Blue in 50% methanol, 10% acetic acid for 2 h at 45° C and the gels were destained initially in the same solvent and then in 5% methanol, 10% acetic acid also at 45° C.

Twitch time

The isometric contraction data were similar to those reported earlier[1,3,9] and showed that the twitch myogram of a cross-reinnervated FHL muscle slowed so that it came to resemble that of a normal or self-reinnervated soleus muscle and *vice versa*.

As has been reported previously[3], the increase in the twitch time of cross-reinnervated fast-twitch muscles is more marked than the decrease in the twitch time of cross-reinnervated soleus muscles, and this observation was confirmed in the present series with one exception. The cross-reinnervated soleus muscle of the experiment of May 18 showed a more marked speeding of its isometric twitch time than we have seen in any animal before or since. The normal (right) soleus of this cat had a contraction time of 87 ms and the cross-reinnervated (left) a contraction time of 25 ms. Because of the unexpectedness of this observation physiological studies were concentrated on the soleus muscles of this cat, and isotonic results were not obtained on either the normal or the cross-reinnervated FHL muscle.

The isotonic contraction results showed a reduction in the maximum shortening velocity of cross-reinnervated fast-twitch muscles as has been reported earlier[7]. But, contrary to this same report[7], we observed in the present experiments (and again particularly in the cat of May 18) a marked increase in the maximum shortening velocity of the cross-reinnervated soleus muscles (see Tables 1 and 2).

Table 1 Characteristics of Normal and Cross-reinnervated FHL and SOL Muscles from May 18 Cat

	N-FHL	X-FHL	N-SOL	X-SOL
* TP (ms)	29	54	87	25
† V_0	—	—	4.82	11.6
‡ Soret band	0.50	0.99	1.0	0.56
§ Rate of fluorescence change (Fig. 1) (s^{-1})	5.6 (3.3)	2.0 (1.2)	1.7 (1.0)	5.0 (2.9)
‖ Mg^{2+}-ATPase (s^{-1})	0.053 (2.9)	0.019 (1.05)	0.018 (1.0)	0.035 (1.95)
¶ Actin-activated Mg^{2+}-ATPase (s^{-1})	2.7 (10.0)	—	0.27 (1.0)	2.5 (9.3)

* TP, time to peak of isometric twitch (the time from the start of contraction until peak tension development).

† V_0, maximum unloaded shortening (muscle fibre lengths s^{-1}).

‡ Soret band, relative absorption (λ_{max} 417 nm) of haem proteins in the first extract of the blended muscle (see preparative procedures)[28,29].

§ Rate of fluorescence change was the observed rate when 12.5 ATP was mixed with the myosins as illustrated in Fig. 1.

‖ Mg^{2+}-dependent myosin ATPase activity using 2.5 mg [illegible] myosin measured in 0.6 M KCl, 5 mM $MgCl_2$, 50 µM dithiothreitol, 0.5 mM EGTA at pH 8.0 and 25° C initiated by addition of 0.4 mM ATP, using a radiometer pH-stat.

¶ Actin-activated Mg^{2+}-dependent myosin ATPase activity using 0.02–0.2 mg ml^{-1} myosin measured in 0.67 mM ATP, 23 mM KCl, 1 mM $MgCl_2$, 6.7 µM $CaCl_2$ at pH 8.0 and 22° C in a radiometer pH-stat, initiated by addition of 0.85 mg ml^{-1} actin. Insufficient X-FHL myosin was available to obtain a reliable actin-activated Mg^{2+}-ATPase activity, but such results as were obtained indicated the activity was comparable to that of the normal soleus myosin. The figures in brackets are values relative to the normal soleus.

Myosin activity

Before studying myosins from the cross-reinnervated muscles several control experiments were carried out on preparations of fast-twitch and slow-twitch myosins from normal cat muscles. ATPase activities of myosins isolated from fresh muscles were identical to those of myosins isolated from corresponding muscles stored at −20° C.

A further control experiment concerned the extractability of myosins from different muscles. Normal FHL and soleus muscles well cleaned of connective tissue were mixed in known proportions by weight (3:1, 2:2, 1:3) and the resultant myosins isolated. Kinetic analysis of the pure myosins and the mixtures gave activities which showed that each myosin was extracted essentially in proportion to its original content in the muscle (that is, the myosin from the 2:2 muscle mixture behaved as an equal weight mixture of pure FHL and soleus muscle myosins). This is an important result, since the yields of myosin from the muscles were variable and often rather low, particularly the cross-reinnervated FHL muscles. These low yields occurred with smaller muscles which seemed to contain a proportionally higher content of connective tissue, and whose myosins precipitated less quantitatively in low salt because of their relatively low concentrations.

The kinetic results from the myosins of the May 18 cat are summarised in Fig. 1 and Table 1. (N-FHL, S-FHL, and X-FHL are respectively normal, self-reinnervated and cross-reinnervated flexor hallucis longus muscles; N-SOL, S-SOL and X-SOL, normal, self-reinnervated and cross-reinnervated soleus muscles.) They show that cross-reinnervation has resulted in almost complete cross over from the kinetic viewpoint. This can clearly be seen in Fig. 1 when the observed rates are dependent solely on the type of myosin and not on its total concentration.

The physiological measurements indicated that in other experiments transformation was less complete and this was reflected in the actin activated Mg^{2+}-dependent ATPase

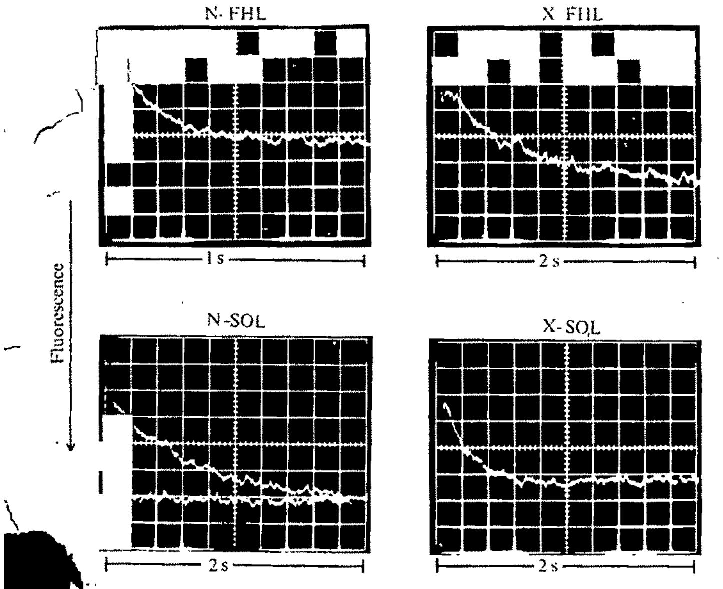

Fig. 1 Stopped-flow spectrophotometric record of the protein fluorescence increase when ATP is mixed with myosin isolated from normal and cross-reinnervated FHL and soleus May 18 cat muscles at 22° C. One syringe of the stopped-flow apparatus contained myosin (0.50 mg ml^{-1}), 2.1 µM in myosin 'heads' and the other 12.5 µM ATP. Both syringes contained 0.5 M KCl, 5 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.2 mM EGTA and 50 mM Tris adjusted to *p*H 8.0 with HCl. Reaction chamber concentrations are recorded. The horizontal trace on the N-SOL myosin photograph was recorded a few seconds after the reaction was initiated and shows the protein fluorescence during steady state ATPase activity. Observed first order rate constants were obtained from the oscilloscope traces, and the mean values of a number of records for each myosin are listed in Table 1. The fluorescence stopped-flow apparatus, built by Professor H. Gutfreund and Dr D. W. Yates, has been described previously[18]. The protein was excited by light at 300 nm and the emitted light between 335 and 375 nm analysed. Measured in this way the percentage increase of protein fluorescence when ATP bound to myosin was 8±2%.

activities (Table 2), where the myosin from cross-reinnervated soleus muscle had $\leq$50% of the control (self-reinnervated) FHL activity. A striking feature of our results is the high activity ratio between the control FHL and soleus myosins from the May 18, August 30 and September 18 cats. (Compare, for example, ratios of approximately 2:1 obtained by Bárány and Close[5].) A lower ratio was obtained from the August 23 cat, where assays were carried out at higher ionic strength and lower actin concentrations. It was important to check whether the assay conditions were close to V_{max} for both proteins with respect to actin. This was shown to be the case by measuring the ATPase activity at several actin concentrations between 0.07 and 1.3 mg ml^{-1}, using the control myosins from the August 30 cat. The S-FHL activity showed a plateau above 0.3 mg/ml actin, while the S-SOL activity was >90% of V_{max} at 1.3 mg ml^{-1} actin. Thus the use of low ionic strength conditions and saturating actin concentration at 25° C produce activity ratios in excess of 10:1 for normal fast-twitch and slow-twitch muscle myosins.

Myosin light chains

Both the physiological and kinetic experiments show that cross-reinnervation can produce a reciprocal transformation of cat fast-twitch and slow-twitch muscles (Table 2), and the myosins were further examined by SDS gel electrophoresis to show the light chain distribution. Figure 2 shows the results for the May 18 cat. The positions of the three light chains characteristic of fast-twitch muscle myosin are shown by the rabbit light chain marker. The myosins from the N-FHL and X-SOL seem identical, so too do the myosins from the S-SOL and X-FHL, though here the number of bands observed in the light chain region is higher than expected. (Compare results with refs 11 and 12 for slow-twitch muscle myosins of the rabbit.) Attempts to eliminate some of these components by further purification of the myosins either by ammonium sulphate precipitation or on DEAE-cellulose columns in pyrophosphate buffers[20] did little to remove the additional bands, though the two fastest bands (Fig. 2*e*, *f*) were somewhat decreased in intensity. Purification by ion exchange chromatography was not used routinely because of the considerable loss of protein during the fractionation and subsequent concentration procedures. In spite of this marked heterogeneity in the light chain band pattern, the myosins from N-SOL and X-FHL were indistinguishable from each other in the light chain region and showed significant differences when compared with the myosins from S-FHL and X-SOL muscles. Similar observations were made for the other cats, showing the cross-reinnervation results in the production of myosins whose light chain mobilities on SDS gels are consistent with a cross over of myosin isoenzyme production.

Table 2 Characteristics of Muscles and Myosins from Cross-reinnervated Cats

August 23 cat	S-FHL	X-FHL	S-SOL	X-SOL
TP	30	51	75	37
V_0	16.3	6.51	6.27	11.1
Actin-activated Mg^{2+}-ATPase (s^{-1})	0.72	0.31	0.19	0.37
Physiological conversion		0.96		0.48
Biochemical conversion		0.77		0.34
August 30 cat				
TP	24.5	52	77	39
V_0	10.8	7.28	6.45	8.43
Actin-activated Mg^{2+}-ATPase (s^{-1})	3.86	0.83	0.20	0.88
Physiological conversion		0.81		0.46
Biochemical conversion		0.83		0.35
September 18 cat				
TP (ms)	31	58	95	45
V_0	13.6	7.58	5.07	8.34
Actin-activated Mg^{2+} ATPase (s^{-1})	2.12	0.71	0.20	0.50
Physiological conversion		0.71		0.38
Biochemical conversion		0.74		0.16

Definitions of TP and V_0 are given in the legend to Table 1. The physiological and biochemical conversions are estimates of the degree of conversion[8] calculated from the V_0 and actin-activated ATPase activities respectively. The physiological conversion for

$$\text{soleus} = \frac{[V_0(\text{X-SOL}) - V_0(\text{S-SOL})]}{[V_0(\text{S-FHL}) - V_0(\text{S-SOL})]}$$

$$\text{and for FHL} = \frac{[V_0(\text{S-FHL}) - V_0(\text{X-FHL})]}{[V_0(\text{S-FHL}) - V_0(\text{S-SOL})]}$$

For the September 18 and August 30 cats the actin-activated Mg^{2+}-dependent ATPase activities were measured using 0.2–1.6 mg ml^{-1} myosin under conditions described in Table 1. The actin concentration was 1.3 mg ml^{-1} throughout. The KCl concentration in the experiments with the August 30 cat was 19 mM instead of 23 mM. For the August 23 cat the assays were carried out using a 2 : 1 (w/w) ratio of actin to myosin and the assay conditions were 30 mM KCl, 2 mM $MgCl_2$, 0.5 mM ATP at *p*H 7.6.

The results described above show a correlation between the physiological changes that accompany cross-reinnervation of cat fast-twitch and slow-twitch muscles and both the kinetic and structural properties of the myosins obtained from these muscles. They confirm and amplify the results of Bárány and Close[5] with the rat and Samaha *et al.*[21] and Buller *et al.*[8] with the cat. In particular the experiment of May 18 demonstrates that in the cat an effectively complete cross over can occur not only in the actin activated Mg^{2+}-dependent ATPase activi-

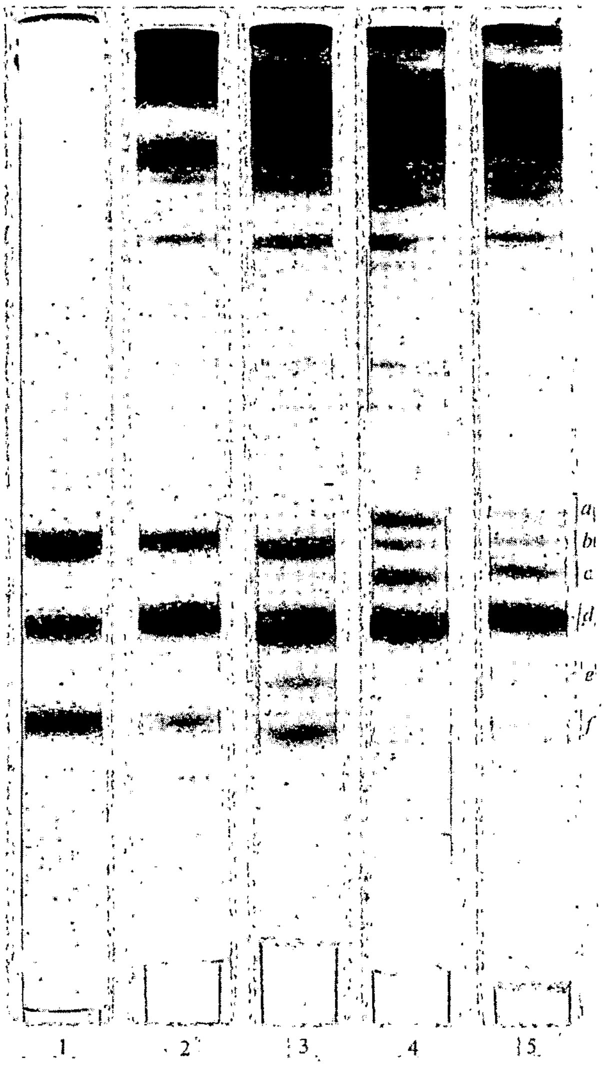

Fig. 2 Polyacrylamide gel electrophoresis of myosins from the May 18 cat run in the presence of SDS on 10% gels. 1, Rabbit myosin light chains; 2, N-FHL; 3, X-SOL; 4, N-SOL; 5, X-FHL. The letters *a–e* denote the light chain bands in the slow-twitch muscle myosin.

ties of the myosins (see ref. 8), but also in the light chain pattern observed by SDS gel electrophoresis of the myosins.

The biochemical methods used in this work for estimating actin-activated ATPase activities have differed somewhat from those described previously[5,8]. Other authors have attempted to retain conditions approximating those found *in vivo* and the actomyosin composition has reflected that observed in myofibrils, whereas we have used techniques to exhibit the maximum differences between the myosins in a variety of kinetic assays which are nevertheless related to important activities *in vivo*. Although attempts to mimic the *in vivo* situation inevitably fall far short of exactness, we see no reason to prefer one set of data to the other. On the other hand we would certainly not claim that our kinetic techniques provide a more precise indication of the *in vivo* situation. In particular we see nothing in the present results to invalidate the Bárány hypothesis[6], though the failure to obtain quantitatively similar ratios for the physiological and biochemical conversions (see Table 2) suggests that factors other than myosin ATPase activity may also play a part in determining a muscle's maximum shortening velocity. Previous reports[5,8] have also contained data on the Ca^{2+}-dependent ATPase activities of the myosins from cross-reinnervated muscles and similar assays were carried out on our myosin preparations, but the results did not show such dramatic differences between the fast-twitch and slow-twitch myosins as are recorded in Tables 1 and 2, and the cross-reinnervated muscle myosins were always intermediate in value between the control activities. This kinetic assay is particularly sensitive to modification of the myosin, for example, through thiol oxidation, and we are inclined to agree with Bárány and Close[5] that it is a less reliable method for distinguishing myosins from cross-reinnervated muscles. Furthermore, this assay has no physiological significance since the role of calcium *in vivo* is regulatory[22].

The complexity of the light chain gel pattern observed in Fig. 2 for cat myosins is greater than that reported for rabbit myosins. Both N-FHL and X-SOL myosins show additional minor bands when compared with rabbit light chains. Control preparations of rabbit soleus myosin show two bands whose mobilities correspond to molecular weights of 27,000 and 18,000[11,12,23] and our observations agree wi[illegible] these. Large scale preparations of myosin from normal [illegible] soleus muscles have, however, always given a pair of ban[illegible] in the 27,000 dalton region corresponding to *a* and *b* in Fig. 2, together wth a strong third band equivalent to *d*. Increased heterogeneity in the light chain region invariably occurred when the light chains were isolated from cat soleus myosin, yielding the additional bands (*c*, *e* and *f*) seen in Fig. 2 for the N-SOL and X-FHL myosins. These additional bands probably arise as a result of proteolytic degradation of the cat myosin.

The significance of the gels shown in Fig. 2 is to demonstrate the overall similarity between myosins from N-SOL and X-FHL muscles and to distinguish these from the N-FHL and X-SOL myosins. This can be most easily appreciated by the presence or absence of band *a*, which is characteristic of slow-twitch muscle myosin. Bands *b*, *d*, *e* and *f* cannot be distinguished by their mobilities alone from correspondin bands in the fast-twitch muscle myosins and, although th presence of bands *e* and *f* in N-SOL and X-FHL myosi may be attributed to proteolytic degradation, the presence of both myosin isoenzymes in the X-FHL cannot be ruled out. Unequivocal assignment of the proteins in these bands can only be made after further analysis of the light chains involved, and current studies on the thiol peptides present in the different light chains should provide unambiguous evidence for the identity of each component.

Other observations indicate that it is not only the light chain components that change following cross-reinnervation. Evidence has been adduced from the content of methylated histidine and lysine that the heavy chains of myosin also change in response to neural influence, and these preliminary studies[24] are supported by gel electrophoretic studies of heavy meromyosins produced from cross-reinnervated rat muscles[25].

All these results indicate that the myosin phenotype is controlled by the nervous system. At the present time it is not clear whether this control is exercised exclusively by the pattern of nerve impulses reaching the muscle, or whether other factors such as a trophic (non-impulse) influence also play a part. That the former is of considerable importanc has recently been demonstrated by experiments in whic myosin light chains characteristic of slow muscle have bee synthesised in response to long-term stimulation of rabbit fast-twitch muscle[26]. It has also been shown that long-term stimulation of cat fast-twitch muscle results in a marked decrease of the muscle's maximum shortening velocity[27]. These observations, together with the present results, certainly suggest that if an altered activation of muscle occurs following cross-reinnervation it might play a major role in the mechanical and biochemical changes which occur. It would be interesting to know if the electromyogram activity of muscles does change following cross-reinnervation.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Spectroscopic Variations in Cyg X-2

THE X-ray source Cyg X-2 has been optically identified with a fifteenth magnitude blue object[1]. Initial spectroscopic observations[2-4] revealed the presence of broad Balmer absorption lines, a narrow, strong Ca II K line and a narrow emission line of He II λ4,686. Radial velocity variations were evident, with the indication that the emission and absorption line velocities varied 180° out of phase. Photometric monitoring[4] showed the object to vary erratically on a time scale of minutes. Later spectroscopic observations[5,6] confirmed the changes in radial velocity, but no certain evidence of binary motion could be demonstrated. A more recent spectroscopic observation[7] showed the He II emission to have developed a P-Cygni profile, and emission at $\lambda\lambda$4,640–50 due to C III–N III was present. I report here the results of additional spectroscopic observations of this object.

Spectrograms were obtained on 1972 July 13.340, 1972 July 14.417 and 1973 July 9.367 UT with the 82-inch (207-cm) and 107-inch (272-cm) reflectors at McDonald Observatory. The first two spectra cover the wavelength region from $\lambda\lambda$4,400 to 6,700 at a dispersion of 210 Å mm^{-1}. The third spectrogram covers the wavelength region from $\lambda\lambda$3,800 to 5,700 at a dispersion of 115 Å mm^{-1}. Both 1972 spectra show the He II and C III–N III emission features and P-Cygni structure is evident in the helium line. The radial velocity of the He II emission is -400 km s^{-1} for both spectra. This same velocity was observed on a spectrogram obtained the previous year[7].

The spectrogram obtained in July 1973 shows a strong Ca II K line, indicating a spectral type of F5 or later. The strength of the Balmer absorption lines, however, indicates a spectral type of A5 or earlier. The C III–N III emission is no longer present. The He II emission line is present, but with no evidence of P-Cygni structure. The measured radial velocity of the He II feature is -390 km s^{-1}. It seems that Cyg X-2 no longer exhibits large radial velocity variations. It should be noted that Cyg X-2 was monitored photometrically in 1971 and 1972 and no rapid variability was seen (P. Vanden Bout, private communication). The above data and the report[8] of radio variability in Cyg X-2 suggest that further optical studies are sorely needed.

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Tungus Event Revisited

THE suggestion of Jackson and Ryan[1] that a "mini" black hole was responsible for the Tungus Event of 1908 is both imaginative and intriguing. Unfortunately, this miniature, hypothetical object cannot account for all the important phenomena known to accompany the event. On the same night of the Tunguska Fall, and for several subsequent nights, the Tungus and adjacent regions experienced abnormally bright skies[2]. Inhabitants of the entire Northern Hemisphere recorded anomalous extinction of atmospheric light subsequent to the fall[2].

Investigation of the soil samples in the Tungus area revealed numerous small magnetic globules with high nickel content. Although the high nickel content affirms that the globules are of extraterrestrial origin, this information alone does not insure that they originated with the Tungus Event. Most soil samples collected at random over the globe will contain similar cosmic dust. The spatial pattern of the globules collected in the Tungus, however, shows that the cosmic dust most likely originated from a massive meteoritic body of dimensions vastly greater than a few Ångstroms. Furthermore, this body had a trajectory similar to that of the Tunguska meteorite[3]. In addition to the magnetic globules, field workers in the area found fused silicate-magnetite spherules near the Tunguska epicentre. This type of cosmic remnant has been found nowhere else in the world[4].

All these data support the hypotheses that the Tunguska Event was caused by a comet with a head composed of iron and silicate fragments cemented together by frozen volatiles. On contact with the Earth's atmosphere, the comet exploded, scattering dust and fragments over the landscape and into the atmosphere, leaving no significant crater.

Although other phenomena may possibly be invoked to account for the Tunguska meteorite, it seems that the black hole is eclipsed by a comet. Of course this discussion does not preclude the possibility that a black hole comprised the nucleus of the comet or that black holes frequently may be the agent's condensing the materials in such bodies.

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Generalisation of Hubble's Law

RECENTLY, several new efforts to improve the upper bound on the possible rest mass (m_0) of the photon have been made[1-3]. The theoretical basis is provided by Proca's equations[1]

$$(\Box + \mu^2)A_K = (4\pi/c)J_K \tag{1}$$

where charge is assumed conserved and $\mu = m_0c/\hbar$. The bounds vary from 2×10^{-43} g to 3×10^{-56} g. As Yourgrau and Woodward[4] point out, however, a finite mass is implicit in a 'tired light' interpretation of the cosmological redshift. When equation (1) is combined with Hubble's law[5]

$$d\nu/dl = -H\nu/c \tag{2}$$

it yields $\mu = H/2c$ and hence $m_0 < 10^{-63}$ g. Here ν is the photon frequency, l the distance traversed and H is Hubble's constant. In view of this limit a direct demonstration of the existence of a finite photon rest mass does not seem practical at present.

Photon-photon interaction has been suggested[6-8] as the 'tired light' mechanism involved. Although this does imply a finite photon rest mass serious objections have been raised on both experimental[9] and theoretical[10,11] grounds.

A direct local laboratory measurement of a 'tired light' effect requires a precision of about 1 part in 10^{18} per light s. That is well beyond present capabilities. As we shall indicate, however, equation (2), when generalised, implies a corollary 'tired body' effect which may be observable in a local system.

To see this, one has only to multiply equation (2) by Planck's constant (h).

$$d(h\nu)/dl = d(h\nu/c)/(dl/c) = -H(h\nu/c) \tag{3}$$

This immediately expresses the change of both the momentum and the energy of the photon as it moves along its path. Both of these relations can be combined into the four-vector form which constitutes the generalised Hubble's law (see ref. 12; a restricted form of equation (4) is given here).

$$dP_K/d\tau = -HP_K \tag{4}$$

The space terms yield the loss of momentum per unit time and the fourth component the loss of energy per unit length. Thus it seems from equation (4) that a non-relativistic body moving with momentum $\bar{P}$ will experience a 'tired body' force.

$$\bar{F} = -H\bar{P} \tag{5}$$

Since bodies in linear motion present the same observational difficulties as does light we turn to rotational motion. From equation (5) we see that the rate of loss of angular momentum L in a rotating system is given by

$$\dot{\bar{L}} = -HL \tag{6}$$

As an application, we can take the Earth-Moon system. (the basis of fossil data we have shown that its angular mome tum has apparently been continually lost over the past 1.75×1 yr[13]. The total loss over this period is about 5% of the present value. Calculation of H from equation (6) gives a value of about 10^{-18} s^{-1} as compared to the latest reported value[14] of $1.3^{+0.8}_{-0.4}\times10^{-18}$ s^{-1}. Details of this calculation are given elsewhere[12].

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BIOLOGICAL SCIENCES

Orthotopic Heart Transplantation in the Pig

THERE is only one report of orthotopic heart grafting in the pig[1]; the longest survivor lived for 11 d. Heart-lung bypass in this species has proved to be difficult[2,3]. Our previous studies of organ grafting in pigs made the study of the orthotopically grafted heart interesting to us.

Landrace or large white/landrace littermate pigs, of either sex, weighing 20 to 30 kg were used as donor and recipient. Anaesthesia was with halothane and endotracheal anaesthesia as described for liver allografting[4]. The animals breathed spontaneously until the chest was opened, when the lungs were ventilated mechanically.

With the recipient pig lying on its back, incisions 8 cm long were made in the right iliac fossa and in the neck along each side of the trachea. On the left side an intravenous (i.v.) infusion was set up in the subclavian vein. Arterial pressure, arterial blood gases and *p*H were monitored by a cannula in the transverse cervical artery and the central venous pressure was measured by a cannula in the internal jugular vein. Heparin (4,000 IU) was given i.v. and a size 18 FG Bardic venous catheter was inserted into the superior vena cava (SVC) through the right external jugular vein. A 3 mm Bentall arterial cannula was inserted into the arch of the aorta through the right common carotid artery. The inferior vena cava (IVC) was cannulated through the right common iliac vein extraperitoneally with a size 20 Bardic venous catheter. The arterial and venous cannulae were connected to the primed lines of the heart-lung bypass machine. Additional tubing was connected from the machine to a low pressure sucker and a left ventricular vent.

Pancuronium (6 mg) was given i.v. and mechanical ventilation was begun. The chest was opened by a median sternotomy. The right pleural cavity was opened, but an attempt was made to leave the left pleural cavity intact, although this was not always possible. A tape was passed around the SVC caudal to the azygos vein; a similar tape was passed around the IVC and both tapes were threaded through rubber tubing to form snares.

For the bypass, the priming volume was 1,300 ml made up of 300 ml of dextran 110 and 1 l of ACD porcine blood less than 24 h old. Added to the priming fluid were 0.5 g potassium chloride, 1 g ampicillin, 4,000 IU heparin, 10 ml sodium bicarbonate (8.4%) and 4 ml calcium chloride (10%). The venous lines passed to two cardiotomy bags from which blood was pumped by a Sarne pump through a heat exchanger and then into a Travenol membrane oxygenator, a filter and then by a second Sarne pump to the arterial line.

The donor pig was anaesthetised by the same technique as the recipient. It lay on its back on the operating table with its left chest rotated uppermost; 3,000 IU of heparin was given i.v. and 6 mg of pancuronium. The lungs were then mechanically ventilated. The abdomen was opened by a midline incision and the abdominal aorta cannulated and connected to an ACD blood collecting bag. Whilst the animal was exsanguinated, the chest was opened on the left side in the fifth interspace, the sternum was divided obliquely and the right chest opened in the fourth space. The pericardium was opened, and the heart removed by first dividing the aorta and pulmonary artery 2 cm from their origins and then dividing the pulmonary veins and venae cavae close to the heart. The heart was inserted into normal saline at 10° C.

For the insertion of the graft, partial bypass was commenced on the recipient. A vent was inserted into the apex of the left ventricle of the donor heart and secured with a catgut purse-string suture. The caval snares were secured and full bypass started. Lung ventilation was reduced to a minimum but not stopped. The aorta and pulmonary artery were clamped and the heart removed leaving most of the atria intact. The donor heart was now removed from saline and the aorta was anastomosed to that of the recipient with a running 4/0 silk suture. Air was expelled from the aorta and the clamp was removed from the recipient aorta and pulmonary artery permitting coronary perfusion. The coronary sinus effluent and blood from the cut hemiazygos vein were returned to the bypass reservoirs via the low pressure sucker. The donor atria and atrial septum were sutured to the recipient atria with running 3/0 mersilene sutures which included the recipient atrial appendages. The pulmonary arterial anastomosis was now completed with a 4/0 running suture and the caval snares were removed. If sinus rhythm had not by now returned spontaneously and there was coarse fibrillation, the heart was defibrillated with a DC shock of 15 joules. If the myocardial tone was poor, 1 ml of 1/10,000 adrenaline was injected into the aorta before defibrillation. The bypass flow was gradually reduced as the donor heart took over the circulation and normal lung ventilation was resumed. Incisions were made in the pericardium to prevent tamponade and a patch of pericardium taken from the donor was sutured to the recipient pericardium to allow for cardiac dilation. The left ventricular vent was clamped and removed after securing a purse-string suture around its site of entry.

Table 1 Pigs with Orthotopic Heart Grafts

Number	Survival	Cause of death if known
1	2 h	Pulmonary oedema
2	2 h	Haemorrhage
3	4 h	
4	2.5 h	
5	7 h	
6	8 h	
7	12 h	Hyperthermia
8	12 h	
9	12 h	
10	2.5 d	
11	5 d	Rejection
12	5 d	Rejection
13	6 d	Rejection
14	18 d	Rejection
15	4.5 months	Rejection
16	4 months	Alive

When bypass was stopped and blood loss was minimal, the heparin was reversed with protamine sulphate given i.v. and fresh donor blood was infused to maintain a central venous pressure (CVP) of 4 to 10 cm of saline. Dipyridamole (Persantin, 10 mg), deslanoside (Cedilanid, 0.4 mg), calcium gluconate (1 g) and sodium bicarbonate (20 mEq), were infused i.v., and if the blood pressure fell 0.5 mg isoprenaline was given. An Argyle chest drain was inserted by the right ninth interspace. If the left pleural cavity was opened, a similar drain was inserted on the left. The bypass cannulae were removed, the right iliac fossa and neck wounds were closed. The chest was closed with trans-sternal nylon interrupted sutures, continuous nylon to approximate the muscles and subcutaneous tissue and chromic gut for the skin.

The three cannulae on the left side of the neck were left in place for i.v. infusion and monitoring of the central venous pressure, arterial pressure, arterial blood gases and *p*H. The chest drain was connected to an underwater seal and anaesthesia was stopped when the effect of the pancuronium had worn off. The animal was sedated with Valium (5 mg) given intramuscularly. When breathing movements were judged to be adequate, mechanical ventilation was stopped, and once spontaneous ventilation seemed to be satisfactory the endotracheal tube was removed, the chest drain was joined to a Heimlich chest drain valve and the pig was placed in a warm bed of straw. The following day the chest drain was removed and the animal was given food and drink *ad libitum*. No immunosuppressive therapy was given.

Sixteen pigs survived the operation, but ten died within the early post-operative period. Six animals survived for between 5 d and 4½ months (Table 1). Five of these died with typical stigmata of rejection consisting of round cell infiltration of the myocardium, oedema and vasculitis. One animal was alive and well 4 months after the operation. Animals surviving after 1 week gained weight and seemed to be healthy until the rapid onset of cardiac failure resulting from rejection.

The technique described here was evolved after much trial and error. Long term survival after bypass with bubble oxygenators was not achieved. Their substitution by the Travenol membrane oxygenator seemed to be the chief factor in achieving success with the bypass. Cullum[1] has described the extreme sensitivity of the porcine heart to ischaemia and advocated completing the aortic anastomosis first to revascularise the myocardium as soon as possible. Using this technique the coronary sinus blood tends to obscure the atrial anastomosis and careful suction is required.

At the end of the operation care of the animal is critical, because the pig recovering from anaesthesia is very intolerant to an endotracheal tube. Once removed, we have not been successful with reintubation. Tachypnoea is marked in the first 24 h and resembles the neonatal respiratory distress syndrome. The loss of surfactant could be a contributory factor. It was hoped that retaining the left pleural cavity intact might reduce respiratory distress but there were long term survivors in animals in whom the left pleural cavity had been opened. Once the animals survived 24 h, recovery was rapid. The pigs resumed normal feeding and seemed to thrive. We were surprised at the long survival in some of our animals. From previous studies by Childe and Morris[5] and ourselves[6] on heterotopic porcine heart grafts, rapid rejection was anticipated and this was observed by Cullum with orthotopic porcine cardiac allografts. We have shown that littermate donor/recipient pigs identical for tissue types, and with negative mixed lymphocyte culture reactions, can tolerate renal allografts for many months[7]. As there is a 1 in 4 chance of tissue type identity between two littermates, it is possible that the long survivors described in this communication received hearts from matched donors. This would indicate that the pig reacts to heart or kidney allografts in a similar manner. Further studies are planned on tissue-typed pigs.

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Genetics of Growth in Axenic Medium of the Cellular Slime Mould *Dictyostelium discoideum*

ALTHOUGH *Dictyostelium discoideum* is normally grown on bacteria[1,2], it is desirable for most biochemical studies to use strains grown in the absence of bacteria (axenically)[3]. Isolation of these strains is laborious, however; for this reason we have investigated the genetic basis of growth in axenic medium and have developed a method for inserting the chromosomes bearing the genes for axenic growth into other non-axenic strains. This is made possible by our finding that genes for axenic growth are located on only two of the seven linkage groups and that chromosomes can be readily exchanged between different strains. Using an improved version of the methods employing parasexuality previously reported[4,5], we have selected axenic resegregants carrying various markers from non-axenic strains.

There are two independently derived strains capable of growth in axenic medium that are commonly used: Ax-2 (ref. 6) (ATCC-24397; derived from strain Ax-1 (ref. 3)) and Ax-3 (ref. 7) (also called A3). Mutants of strain Ax-3 which are temperature sensitive for growth were isolated using a bromodeoxyuridine selection method (R. H. Kessin and P. C. Newell, unpublished results). Non-axenic temperature sensitive strains M28 and TS12 were obtained from Dr E. R. Katz (Division of Biological Sciences, SUNY, Stony Brook, New York, USA). A temperature sensitive strain (Ax-2g88) of Ax-2 was obtained from Drs E. Gingold and J. M. Ashworth (Department of Biochemistry, Leicester University, England).

Temperature sensitive haploid strains to be fused to form temperature resistant diploids were grown in association with *A. aerogenes* for approximately 48 h at 22° C. Cells were collected from pre-aggregation plates, washed in cold water, and two mutants containing non-identical temperature sensitive mutations were shaken in equal numbers (5×10^6 of each) in 1 ml of water, or in some cases potassium phosphate (20 mM, *p*H 6.0–7.5) in a Linbro tissue culture dish (FB-16-24-TC). Shaking was for 15–17 h at 22° C, 150 cycles min^{-1}; under these conditions amoebae form 'balls' of aggregated cells[2]. Controls were conducted in which each parent was incubated separately. After incubation the aggregated cells were separate by vortexing, counted with a haemocytometer, and spread wit *A. aerogenes* on agar plates at the restrictive temperatu (27° C) at 1×10^6 to 3×10^6 amoebae per Petri dish. Temperature resistant diploids were selected at a frequency of about 1 in 10^6 amoebae. Their ploidy was confirmed using genetic markers, spore size, and, in a number of cases, cytological study. Diploids were purified by clonal passage and their spores stored in silica gel at 4° C. It was possible to maintain these diploids by clonal passage; they had a stability similar to that reported by Katz and Sussman[4].

The method used to form diploids described here considerably facilitates parasexual genetics[4] since (1) relatively few cells are used; (2) large numbers of crosses can be done at one time in the Linbro tissue culture dish which has twenty-four wells; (3) the method is quick and simple as the cells are not placed on filters; (4) the medium used for fusion can be varied and many parameters can be tested concurrently; (5) it is possible to fuse not only two non-axenic strains (see Katz and Sussman[4]) but also axenic and non-axenic strains and two axenic strains; (6) aggregateless and other morphological mutants can be fused readily using this method.

Growth in axenic medium of strains Ax-2 and Ax-3 was found to be a recessive character since diploids heterozygous for axenic chromosomes (formed by fusion of an axenic and a non-axenic strain) were phenotypically not able to grow in axenic medium. Using this fact it was possible to select haploid axenic strains by placing diploids heterozygous for axenic genes at a low concentration (10^4–10^5 ml^{-1}) in axenic medium. After a lag of approximately a week, growth of haploids was usually observed. The haploid axenic strain X2 derived from the diploid of M28 and Ax-3tsg1 was selected in this way (Table 1). Haploid axenic strains were also selected using resistance to recessive drug resistance markers. For example X14 was selected on cycloheximide (500 μg ml^{-1}) from the cycloheximide-sensitive diploid of X9 and NP12 (Table 1).

Diploids homozygous for Ax-3 chromosomes have been isolated. These grow axenically, although subculture in axenic medium results in an ever increasing frequency of haploids until only rare diploid cells remain. This reduction in ploidy from diploid to haploid also occurs when these diploids are grown in association with bacteria without cloning. It has been reported previously that ploidy in the diploid strain H-1 changes from diploid to haploid in bacterial culture in the absence of cloning[8,9].

Diploids formed by fusing Ax-2 and Ax-3 haploids grew axenically. Three such independently isolated diploids have been studied; they showed similar growth characteristics in axenic medium to homozygous Ax-3 diploids. Detailed cytological studies on all of these diploids will be reported elsewhere. From these results it seems that the genes involved with growth in axenic medium in the independently isolated strains Ax-2[6]

Table 1 Resegregated Axenic Strains

Parental haploids	Haploid resegregant*	Growth in axenic medium	Growth at 27° C	Brown pigment production	Spore colour	Spore shape	Cycloheximide resistance (500 μg ml^{-1})	Acriflavin resistance (100 μg ml^{-1})	Colony morphology
M28		−	−	+		Round			
NP2		+	−	−		Elliptical			
	X2	+	−	+		Elliptical			
†X9		−	−	+	White		+		
‡NP12		+	−	−	Yellow		−		
	X14	+	+	+	Yellow		+		
NP29		−	−	+				−	Aberrant aggregation
NP12		+	−	−				+	Normal
	X17	+	+	+				+	Normal

+ Denotes the presence of the characteristic.
* Resegregated strains are called X1 . . . n.
† Strain X9 was selected from the diploid of M28 × NP14.
‡ NP14 and NP12 are acriflavin-resistant strains of TS12 and NP2 respectively; no mutagenesis was used in their isolation.
To aid clarity, characteristics which are identical in both parental strains have been omitted.

[illegible] Ax-3[7] may be identical, or at least that the recessive genes [illegible]ed are the same. We have no evidence yet whether in addition to recessive genes there are dominant genes involved with growth in axenic medium, or indeed how many genes there are. From analysis of segregant classes (to be published elsewhere) it seems, however, that only two linkage groups have genes required for growth in axenic medium. The point to be stressed here is that since ability to grow in axenic medium in strains Ax-2 and Ax-3 is probably not associated with five linkage groups, these linkage groups from non-axenic strains can be readily inserted into axenic strains. By forming appropriate diploids, properties such as cycloheximide resistance, round spore formation, and brown pigment formation have been incorporated into strain Ax-3.

While all of the strains used in these genetic studies are temperature sensitive for growth, it is possible, by using two strains whose temperature sensitive mutations are unlinked, to select temperature resistant (wild type) haploid segregants with various markers. Ax-3 sporulates very poorly at 27° C; this is a serious disadvantage for developmental studies where wild-type and temperature sensitive strains are to be compared. We have selected temperature resistant haploid axenic strains (Ax-3) which show improved sporulation at 27° C. For example, X14 (mentioned previously), Table 1; X17, selected on acriflavin (100 μg ml^{-1}) from the acriflavin sensitive diploid of NP29 and NP12 (Table 1).

Axenic strains grow more slowly on bacteria than non-axenic strains[6]. Some of our temperature sensitive haploid axenics grow very slowly on bacteria, but by selecting haploid axenics with some chromosomes from non-axenics, we have been able to obtain axenic strains with growth rates on bacteria that are much improved (for example, strain X2, Table 1).

While our primary aim is to develop genetic analysis so that factors involved with cell interaction in *D. discoideum* can be studied, strains that are well characterised both genetically and biochemically are necessary. We believe that using the methods discussed here, selection of such strains is facilitated.

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X-ray Studies of the Interaction of CO_2 with Human Deoxyhaemoglobin

In addition to transporting CO_2 indirectly by way of the alkaline Bohr effect, haemoglobin can interact directly with CO_2 to form carbamate residues:

$$RNH_2 + CO_2 \rightleftharpoons RNHCOO^- + H^+$$

This reaction of CO_2 with haemoglobin is oxygen-linked so that at constant *p*H more CO_2 is bound to deoxy- than to oxyhaemoglobin, and conversely the oxygen affinity of haemoglobin is decreased in the presence of CO_2 (refs 1 and 2). The studies of Kilmartin and Rossi-Bernardi[3,4] on horse haemoglobin specifically modified at the N terminal amino groups by reaction with cyanate showed that at constant *p*H CO_2 has no effect on the oxygen affinity of haemoglobin when all four α-amino groups are carbamylated, but that only part of the oxygen-linked effect is inhibited when the α-amino groups of only the α or only the β chains are blocked. This implies that the N-terminal amino groups are solely responsible for the oxygen-linked CO_2 interactions and that this effect is shared between the α and β chains. More recent experiments have confirmed these results for human haemoglobin[5].

The X-ray study described attempts to locate the binding of CO_2 to crystalline human deoxyhaemoglobin directly. Because of unpublished evidence (quoted in ref. 2) that concentrated phosphate solutions "affect the reactions of haemoglobin with CO_2", these experiments were carried out with deoxyhaemoglobin crystals[6] soaked in solutions of 70% 2-methyl-2,4-pentanediol (MPD) to remove the native high salt mother liquor. Two sets of 3.5 Å diffractometer data were collected, one on crystals soaked in 70% MPD+CO_2/bicarbonate buffer at *p*H 7.4 (the CO_2 pressure was 1 atm), and another on crystals soaked in 70% MPD+20 mM bis-Tris buffer at *p*H 7.4. Figure 1 shows the difference electron density, calculated by subtraction of the two sets of data and using the native (high salt) structure factor phases[7]. The expected carbamate group appears as a positive peak, about 3 Å in diameter, immediately adjacent to the α-amino group of the β chains. Next to this positive peak is a large negative peak, which indicates that the native electron density labelled X^- in Fig. 1 has been displaced by the newly formed carbamate ion. The same displacement of X^- was observed in the X-ray studies of 2,3-diphosphoglycerate (DPG)[8] and inositol hexaphosphate (IHP; my unpublished work) binding, corroborating the identification of X^- as an inorganic anion, probably phosphate or sulphate.

Binding of organic phosphates produced marked changes in the conformation of deoxy β subunits (ref. 8 and my unpublished work) but binding of CO_2 does not seem to affect them. No movements can be detected among the several basic groups in the neighbourhood of the α-amino groups that would indicate the formation of strong carbamate salt bridges. Instead, the carbamate ion seems to be taking the place of the inorganic anion, X^-, and simply reduces the high concentration of positive charge at the entrance to the central cavity of the haemoglobin tetramer. Similarly, the environment of glutamate 6β—the site of the mutation in sickle cell haemoglobin, haemoglobin S—is unaffected by carbamate formation. Therefore (like DPG[9,10]) CO_2 should promote sickling at low and medium oxygen saturation levels by stabilising the deoxy conformation, but (unlike DPG[8,10,11]) CO_2 may not influence the gelation of fully deoxygenated haemoglobin S since under these conditions CO_2 would alter neither the quaternary structure of the tetramer nor the tertiary structure of the β subunits. The CO_2 difference map shows no carbamate formation at the N termini of the α chains. The reasons for this are not clear.

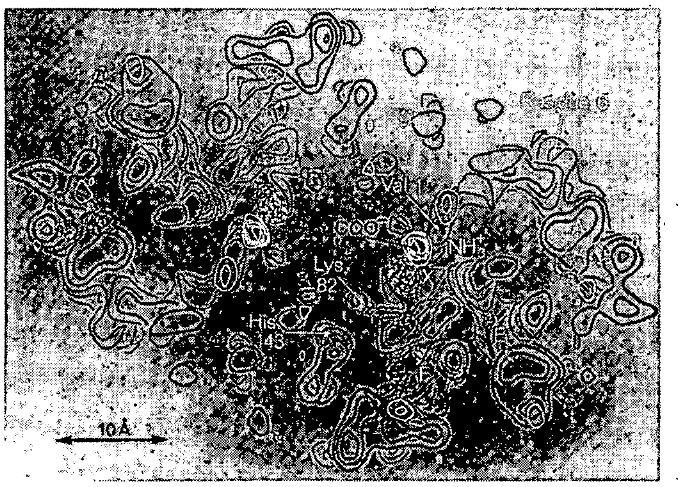

Fig. 1 Fourier map showing the reaction of CO_2 with the N-terminal amino groups of the β chains. Black contours: native human deoxyhaemoglobin electron density map at 3.5 Å resolution (9 sections, −15 to −23 Å). This region includes helices A, E and F and residues Val 1, Glu 6 (the site of the sickle cell mutation), Lys 82 and His 143 of each β chain. These features are related in pairs by the molecular two-fold axis of symmetry. The peak marked X^- has been identified as an inorganic anion (see text). The contours are drawn at intervals of 0.15 e Å^{-3} above the zero level. White contours: difference electron density due to CO_2 binding (3 sections, −18, −19 and −20 Å). Positive difference electron density is marked in solid white contours, negative difference electron density is marked in dashed white contours. The first difference contours are drawn at ±0.0091 e Å^{-3}, the next contours are at intervals of ±0.0045 e Å^{-3}.

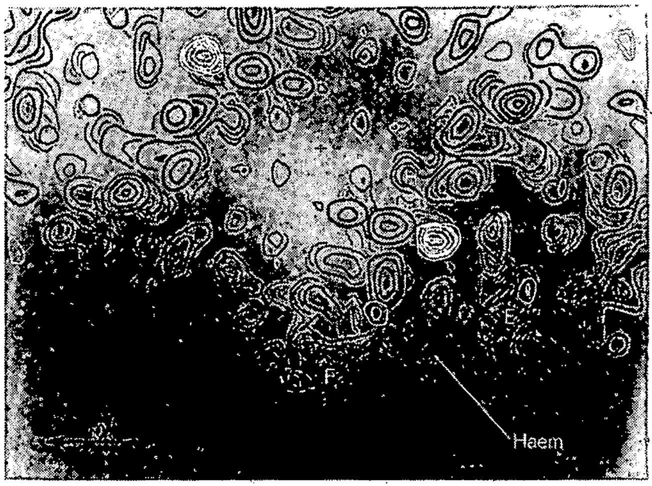

Fig. 2 Fourier map showing the binding of CO_2 to the region of the β-haem group. Black contours: native human deoxyhaemoglobin electron density map at 3.5 Å resolution (7 sections, −8 to −14 Å). This region includes parts of helices E, F and H. White contours: difference electron density due to CO_2 binding (2 sections, −8 and −9 Å) superimposed on th native protein density. Contour levels as in Fig. 1.

There is an unexpected positive difference peak the β haem group (Fig. 2). The atomic model o haemoglobin shows this strong positive peak to be em dded in the centre of a highly hydrophobic hole. The dimensions of the peak are consistent with the binding of CO_2 or HCO_3^-, but the hydrophobic nature of the binding site favours the neutral CO_2 molecule. Since physiological CO_2 pressures do not affect the oxygen affinity of cyanate-blocked haemoglobin[3-5], and I have used nearly twenty times the physiological pressure, the binding of CO_2 at this site may be insignificant at these pressures (40 to 50 mm Hg). If it is significant it should not be oxygen-linked; however, it should be remembered that gases do form non-covalent complexes with haemoglobin[12], and that xenon binds to both the α and β chains at specific hydrophobic sites formed by the A–B and G–H corners[13].

In summary, this X-ray study confirms that CO_2 reacts with the N-terminal amino groups of the β chains to form carbamate ions. It also shows that this reaction displaces an inorganic anion normally bound to the β chain α amino group, but does not cause significant changes in the tertiary structure of the deoxy β subunits. The conditions used in this study of high CO_2 pressure and a low salt MPD environment may, however, have exaggerated a minor effect—the binding of CO_2 in a hydrophobic hole to the rear of the β haem group—and inhibited a major effect—the reaction of CO_2 with the N terminal amino groups of the α chain.

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Cell Shape Regulation and Cell Cycle in Embryonic Lens Cells

EYE lens development in the chicken embryo begins with transformation of the ectoderm destined to become lens from a low cuboidal to a high columnar epithelium. Simultaneously the nuclei pass to the basal poles of the cells. This 'palisading'[1] gives the lens anlage a regular appearance, but individual cells may deviate considerably from he columnar shape, largely because of their replicative ehaviour.

All presumptive lens cells are dividing asynchronously at this stage[2] and show interkinetic nuclear migration[3]. This phenomenon, first described by Sauer[4], has been studied extensively in the neural tube, but occurs in many embryonic epithelia. Cells are anchored at their free apical surfaces by intercellular complexes and contract toward this surface for mitosis. Afterwards, during G_1, they elongate again and their nuclei are displaced toward the basal parts of the cells, where DNA synthesis is initiated. At the end of the S phase the nuclei take the reverse route; cells round up again and a new round of mitosis begins. The position and size of the nucleus therefore can be used to classify cells morphologically according to cell cycle phase (our report in preparation). We describe here that the latter is correlated with the surface to volume ratio of the cells and suggest that cell volume regulation may play a role in cell shaping.

White Leghorn chicken embryos, incubated for 48–55 h, were fixed in glutaraldehyde and osmium tetroxide and embedded in Araldite by routine methods. Frontal sections were cut serially at 0.5 μm and stained with toluidine blue. Invaginating rudiments (stages 14 and 15 according to O'Rahilly and Meyer[5]) were selected and from photomicrographs serial tracings were made of lens cells and nuclei. From planimetric measurements we calculated for each cell the distance from the centre of the nucleus to the basement membrane, nuclear volume, cellular surface area and volume, and cell surface to volume ratio.

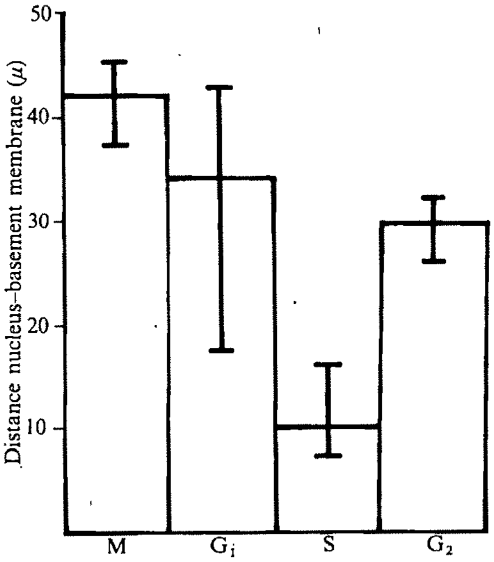

Fig. 1 Histogram indicating the extent of nuclear migration during the replication cycle of cells of the invaginating lens cup. For cells in mitosis the centre of the cell was assumed to correspond with the centre of the nucleus. The vertical bars in this and the following figures indicate the actual range of values; the columns represent the averages of the measurements.

Figure 1 shows that the nuclei migrate up and down more than 40 μm when the tissue is approximately 50 μm tall. When the nuclear membrane reforms after mitosis the nuclear volume is about 70 μm^3. It increases to four times as much for the average G_2 cell. In general, nuclei take up more than a third of the cellular volume during interphase (compare Figs 2 and 4).

Measurements of the cellular surfaces show that the average surface area in G_1 is about half that of mitotic cells. The area then increases with the progression of the cell through the cell cycle, but as the cell approaches division and contracts toward the free tissue surface again, the increase slows down and there may even be a slight decrease of surface area (Fig. 3). The changes in cell volume as a function of cell cycle phase show some intriguing differences from the surface changes. Most notably, the value for the G_1 cell is substantially less than the expected half of the mitotic cell volume (Fig. 4). Cell volume then increases, more or less parallel to the surface area curve, but at the end of the cell cycle when the cell approaches division the volume continues to increase instead of diminishing as the surface area does, and the mitotic cell is on average considerably larger than the G_2 cell. As a result, the surface to volume ratio is smallest during mitosis and largest in G_1, with a gradual decrease during S and G_2 (Fig. 5).

We believe that these observations are germane to an understanding of the structural changes of presumptive lens cells in relation to the cell cycle as well as the developmental stage. The decreasing surface to volume ratio in G_2 indicates that the volume increase outpaces the growth of the surface, suggesting the generation of membrane tensile stresses. Entry into mitosis is accompanied by a further substantial increase in cell volume and a slight decrease in surface area. A sphere has the smallest surface to volume ratio of all possible configurations of a given volume and, moreover, this ratio diminishes with increasing size. Geometry makes it tempting, therefore, to postulate that volume increase

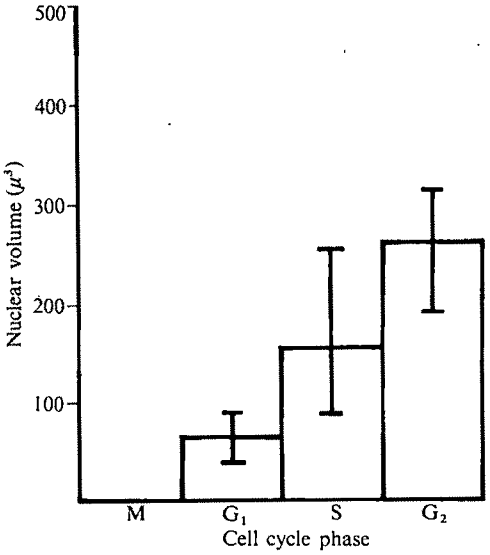

Fig. 2 Cell nuclei become larger with the progression of the cell cycle. Obviously, during division the nuclear membrane has become dissociated and the nuclear volume cannot be measured. Nuclear volumes were determined in the same way as cellular volumes (Fig. 4).

forces the cell to round up in preparation for mitosis. The very rapid change in volume may be due to an influx of fluid from the cellular environment, an explanation compatible with the low density of the mitotic cell, as judged from staining characteristics. These interpretations agree with earlier results: it is well known that cleavage of sea urchin eggs is accompanied by increase of tension in the cell membrane[6], and L cells in culture show appreciable increases in volume and decreases in surface to volume ratio when division begins[7].

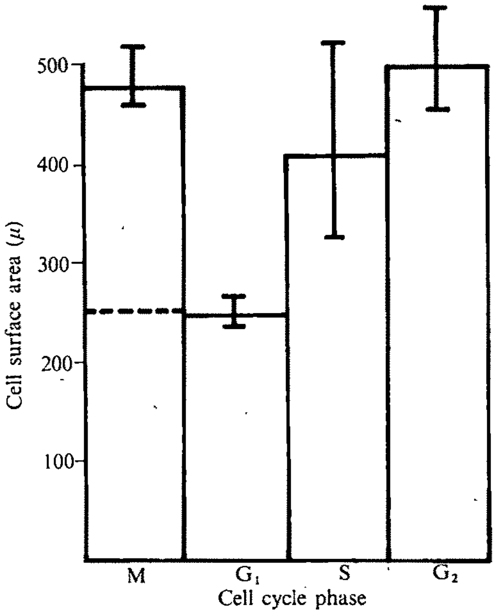

Fig. 3 The average cell surface as a function of cell cycle phase. The surface of each cell was calculated by summating all measured outlines of the cell in serial photomicrographs and taking into account the magnification factor and the thickness of the sections. Electron microscopy indicates that the surface of lens cells at this stage is very regular. Therefore, the outline of the cell at the light microscopic level does in fact represent its surface. Note that the average surface area of the G_1 cell is about the same as half the surface of the mitotic cell (broken line in first column). The surface area becomes larger with the progression of the cell cycle, but appears to diminish somewhat at the end, with the transition from G_2 into M.

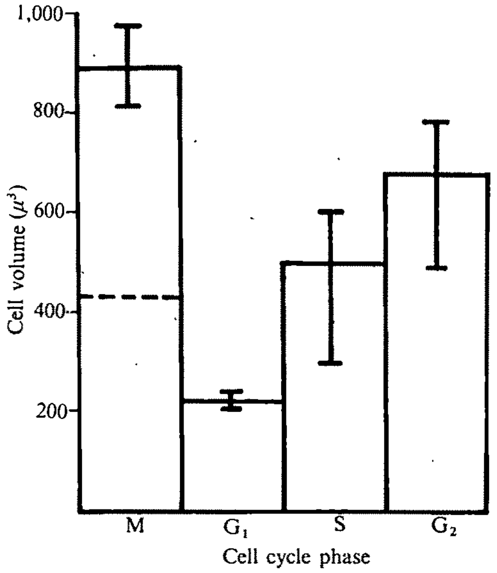

Fig. 4 Cell volume in relation to the cell cycle. The volume was calculated by planimetrically determining the surface of each section through a cell from serial photomicrographs, summating all the areas, and taking into account the magnification and the section thickness. In striking contrast with the surface area changes (Fig. 3) the G_1 cell volume is much smaller than that of the halved mitotic cell. The volume increases continuously over the cell cycle, and the dividing cell is clearly larger than the G_2 cell.

With the transition from mitosis to G_1, the opposite occurs. The division of a sphere into two smaller spheres requires more surface area, relative to the same total volume. This requirement increases when the cell elongates in G_1. Theoretically, the cell can only attain this change in structure by increasing its surface or decreasing its volume. Apparently the latter is favoured in this system, the average G_1 cell volume being considerably less than the expected half of the mitotic cell volume. Because G_1 cells stain very intensely we believe that loss of cytoplasmic fluid may be involved. Whether active transport phenomena are responsible remains to be determined.

Lens placode formation is characterised by cellular elongation and it seemed possible that the extent of the latter is regulated by controlling the volume, apparently lost by the post-mitotic cell. Preliminary work indicates that this is not the case. Throughout early lens development the changes in cell volume and surface in relation to the cell cycle are comparable with those described here for the invaginating rudiment. Thus, we hypothesise that the high surface to volume ratio of the G_1 cells allows it to assume various shapes, including the columnar one. Conversely, the diminishing of the ratio in G_2 limits this freedom and pushes the cell towards a spherical form. Cell volume decrease in G_1 permits the cell to elongate, but it does not instruct it to do so; other forces must cause the elongated shape to be favoured[8].

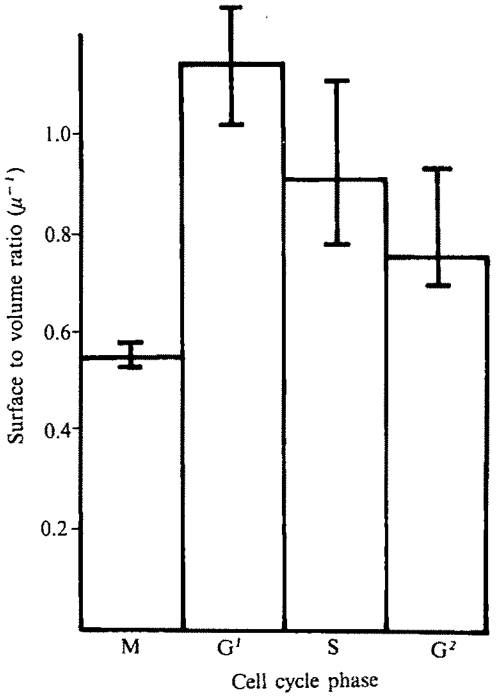

Fig. 5 The surface to volume ratio decreases from high values for G_1 to the lowest ones for mitotic cells, indicative of a progressive limitation of the theoretical freedom that the cell has to assume various morphological configurations. Geometric laws dictate that a structure with a high surface to volume ratio can have any number of shapes, while a low ratio has to be associated with a spheroid form.

Our observations indicate that regulation of cell volume may well delineate the options open to a cell in the determination of its shape. Except for Cone's work[7], this aspect has received little attention. If the volume adjustments found here turn out to involve active transport mechanisms, as we suspect, the associated osmotic changes may well be important in the control of mitosis[7] and DNA synthesis[8]. Our methodology should be applicable to other systems to facilitate classification of cells according to cell cycle phase without reference to synchronisation and (or) isolation of cell populations.

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Precursor 16S RNA in Active 30S Ribosomes

BIOGENESIS of bacterial ribosomes proceeds through the formation of precursor particles[1–4], consisting of an incomplete set of ribosomal protein[5] and of RNA molecules that are longer and less methylated than mature RNA[4,6,7]. RNA maturation is commonly considered to be a part of the ribosome maturation process. Here we report that newly formed 30S ribosomes can enter polyribosomes while they still contain precursor RNA. Maturation of ribosomal RNA might be completed not before, but after, ribosomes have interacted with messenger RNA.

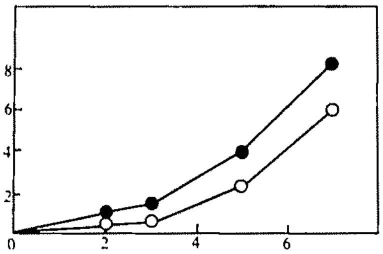

Fig. 1 ^{3}H-uracil uptake in m16S RNA and 30S particles. A culture of *Escherichia coli* D10 in medium MS9 (ref. 9) supplemented with 0.5% casa-amino acids (doubling time 32 min) was labelled with ^{14}C-uracil (0.1 μC ml^{-1}). After two generations, ^{3}H-uracil was added (5 μC ml^{-1}). At indicated times, samples were rapidly chilled and lysed as previously described[10]. A portion of each lysate was centrifuged through a 10% to 30% sucrose gradient in 10^{-4} M MgCl$_2$, 10^{-2} M Tris-HCl, *p*H 7.8, at 40,000 r.p.m. for 8 h to display 30S ribosomes and precursor particles. The peak fraction of the 30S region of each gradient was diluted with three volumes of 10^{-4} M MgCl$_2$ and centrifuged through a second sucrose gradient to resolve 30S ribosomes from precursor particles completely. The ratios of ^{3}H to ^{14}C counts in 30S ribosomes are plotted against labelling time (●—●). Another portion of each lysate was treated with phenol and sodium dodecyl sulphate to extract the RNA. After ethanol precipitation, the RNA was electrophoresed for 6 h at 5 mA per tube on 3.6% polyacrylamide gels prepared as previously described[10]. The ^{3}H to ^{14}C ratios in m16S RNA were multiplied by a factor 1.5 and then plotted against time (○—○). This correction was necessary to obtain comparison with 30S ribosome ratios, since different conditions were used for counting sucrose gradient and gel fractions. ^{3}H and ^{14}C counting efficiency was controlled for each experiment by including an extra gradient with 30S ribosomes uniformly labelled with ^{3}H and ^{14}C uracil, and an extra gel with 16S RNA extracted from the same 30S particles.

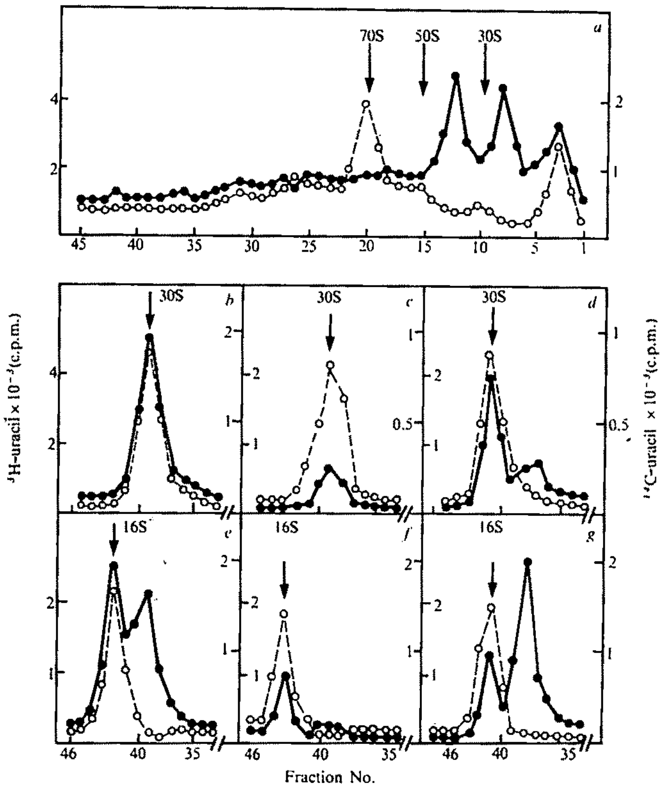

Fig. 2 Newly formed RNA in native and derived 30S subunits. *a*, A lysate of cells labelled with ^{14}C-uracil for two generations and with ^{3}H-uracil for 3 min, as in Fig. 1, was centrifuged on a sucrose gradient in 10^{-2} M MgCl$_2$, 10^{-2} M Tris-HCl, *p*H 7.8, for 2 h at 40,000 r.p.m. to display free and messenger-bound ribosomes. Fractions 25–42 were pooled and dialysed at 2° C against 10^{-4} M MgCl$_2$, 10^{-2} M Tris-HCl, *p*H 7.8. Ribosomal subunits were collected by centrifugation and resuspended in 0.5 ml of the dialysis buffer. This sample, as well as fractions 20 and 10 of the original gradient, were centrifuged on three parallel gradients in 10^{-4} M MgCl$_2$ for 8 h at 40,000 r.p.m. to display 30S subunits. *b*, 30S derived from polyribosomes (fractions 25–42 of *a*); *c*, 30S derived from 70S (fraction 20); *d*, native 30S (from fraction 10). From the peak fraction of each of these three gradients RNA was extracted and analysed by gel electrophoresis as in Fig. 1. *e*, RNA from polyribosomal 30S; *f*, RNA from 30S derived from 70S; *g*, RNA from native 30S. The top of gradients and of gels is at the right. ●, ^{3}H-uracil c.p.m.; ○, ^{14}C-uracil c.p.m.

As we have briefly noted elsewhere[8], conversion of 17S and 16.5S RNA (p16S) into mature 16S RNA (m16S) does not occur at the same time as that of precursor particles into 30S ribosomes. In the experiment summarised in Fig. 1, we have followed the entry of ^{3}H-uracil into m16S RNA and 30S particles during continuous labelling. A delay of 1 to 2 min in m16S RNA labelling was found by comparison with 30S particles.

This suggested that newly formed RNA is incorporated in particles sedimenting as 30S while it is still immature. These particles might be precursors not resolved from mature ribosomes on sucrose gradient, like those recently described by Lindahl[11]. When we examined the distribution of newly synthesised RNA in various ribosomal fractions we found, however, that p16S was present not only in native 30S ribosomes[12], but also in 30S subunits derived from polyribosomes (Fig. 2). The ratio between mature and immature RNA was

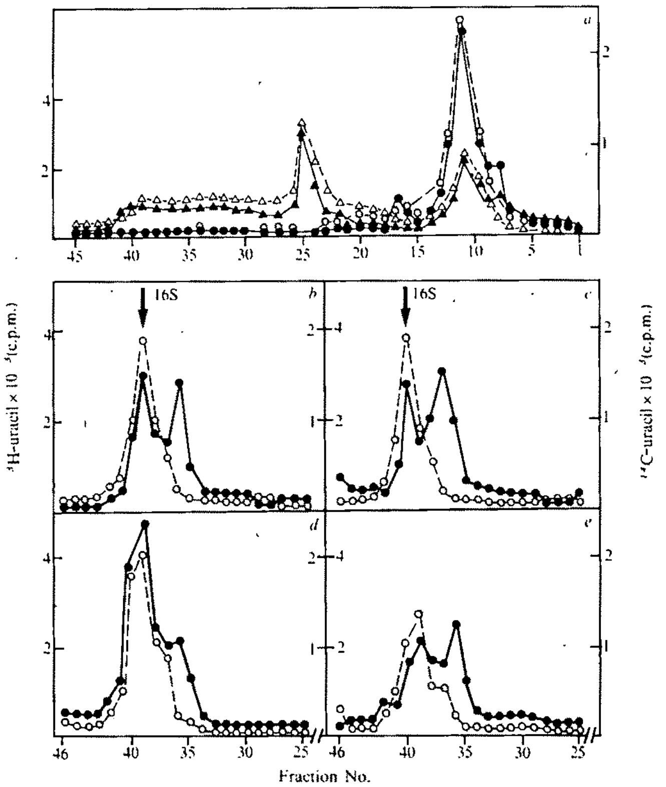

Fig. 3 Formation *in vitro* of polyribosomes from native subunits and T4 RNA. Native 30S particles were isolated by sucrose gradient from a lysate of cells prelabelled with ^{14}C-uracil and labelled with ^{3}H-uracil for 3 min. The labelled particles were mixed with a crude extract (S30) prepared from unlabelled cells, and incubated in conditions of protein synthesis with or without T4 RNA[10]. S30 had been preincubated at 37° C for 10 min. After 2 min of incubation chloramphenicol was added (200 μg ml^{-1}) and the mixture was centrifuged on a sucrose gradient in 8 mM $MgCl_2$, 10 mM Tris-HCl, *p*H 7.8, for 2 h at 40,000 r.p.m. *a*, Gradient distribution of 30S incubated for 2 min with (▲, ^{3}H; △, ^{14}C) and without (●, ^{3}H; ○, ^{14}C) T4 RNA. RNA was extracted from fractions 26–40 and from fraction 10 of the first of the two gradients. *b* and *c* show the gel analysis of these two preparations of RNA, in that order. The top of gels is at the right. The same analysis was made on 30S ribosomes incubated with T4 RNA for 4 min. The gradient is not shown. In *d* and *e*, gel patterns are given for RNA extracted from polyribosomes and free 30S.

different in the two fractions. After 3 min of labelling, native 30S particles contained most of their ^{3}H label as p16S RNA; 30S derived from polyribosomes contained half of their ^{3}H label as p16S and half as m16S RNA. 30S subunits derived from 70S monosomes contained only 16S RNA, Later the portion of ^{3}H label increased in all fractions.

These results indicate that immature RNA can be incorporated in particles that not only sediment as 30S, but can also enter polyribosomes. The relationship between ribosomal subunits found free in a cell extract and those in polyribosomes and 70S monosomes is not yet completely clear[13]. The ratio of immature to mature RNA in these fractions is, however, consistent with the hypothesis that newly formed 30S ribosomes enter polyribosomes while they still carry p16S RNA and are released after the RNA is converted to m16S. Maturation of the RNA would then occur in polyribosomes.

This interpretation is supported by *in vitro* experiments in which native 30S particles were tested for their ability to respond to natural messenger RNA. Labelled 30S ribosomes were added to a cell crude extract and incubated in conditions of protein synthesis (Fig. 3). In the presence of T4 phage specific RNA, newly formed ^{3}H-labelled 30S particles formed 70S and polyribosomes with the same efficiency as the pre-existing ^{14}C-labelled 30S. After the first 2 min of incubation *in vitro*, the ^{3}H label that had entered polyribosomes was distributed between m16S and p16S RNA in the same proportion as in free 30S particles. After 4 min, the ^{3}H fraction in m16S was higher in polyribosomes than in free subunits. In the absence of T4 RNA, no formation of polyribosomes and no conversion of p16S into m16S RNA were observed under the same conditions. These data confirm that 30S particles containing p16S RNA can enter polyribosomes, and once again suggest that maturation of the RNA occurs preferentially in polyribosomes.

We were unable to test whether the particles containing p16S RNA are fully competent in protein synthesis because we could not separate them from those containing m16S RNA. For the same reason, we could not determine their protein content. Furthermore p16S RNA was identified by gel electrophoresis, but was not characterised further. Therefore the possibility that RNA maturation plays a role in the structural or functional maturation of ribosomes cannot be excluded.

The data presented here, however, suggest an additional possibility, namely that complete RNA maturation may n be required to make a ribosome functional. On the contrar the functioning of a ribosome may be necessary for its RNA to mature. A conformational change of the particle, for example, may be required to expose the RNA to a nuclease attack. In this case splitting of the extra nucleotide sequences from the immature RNA molecules would occur at a rate determined by the chance which newly formed ribosomes had of entering polyribosomes, and therefore could serve to signal how many new ribosomes are needed.

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Antibody-dependent Killing of *Cryptococcus neoformans* by Human Peripheral Blood Mononuclear Cells

CRYPTOCOCCOSIS is a systemic fungus infection caused by the encapsulated yeast-like fungus, *Cryptococcus neoformans*. It has been suggested that alterations in cellular host defence mechanisms increase susceptibility to infection with this organism[1,2]. *In vitro* studies suggest that human peripheral blood neutrophils and monocytes can ingest and kill cryptococci[3,4]. There is, however, a wide range of killing in cells from different normal subjects (21–89%), and no apparent defect in cells from patients with cryptococcosis[3]. This makes it unlikely that peripheral blood neutrophils or monocytes play a decisive role in determining susceptibility to cryptococcosis in individual patients. In other *in vitro* studies, activated macrophages derived from human peripheral blood monocytes not only failed to kill cryptococci, but provided a preferentially favourable medium for intracellular growth of the fungus[5]. These studies, as well as the known histopathology of cryptococcal lesions, suggest that many of the organisms, particularly large capsule forms, are extracellular and so not subject to the intracellular killing mechanisms of phagocytes. Anti-cryptococcal antibody, with or without complement, does not kill cryptococci in the absence of leukocytes[3]. I therefore investigated the possible existence of a non-phagocytic cellular killing mechanism.

Mononuclear cells were separated from heparinised human peripheral blood from normal donors as outlined by Böyum[6]. Final preparations consisted of 20–38% monocytes (by neutral red ingestion), plus 62–80% lymphoid cells of other types. A small capsule isolate of *Cryptococcus neoformans* was cultured 72 h on Sabouraud's agar and washed as previously described[3]. Anti-cryptococcal antibody was raised in rabbits and standardised by slide agglutination as outlined by Wilson *et al.*[7]. The agglutination titre of serum used in these studies was 1 : 512. Control serum was obtained from the same rabbit before development of antibody.

If phagocytosis is blocked, it seems that mononuclear cells can readily kill cryptococci only if anti-cryptococcal antibody is present. Maximum killing was noted after 24 h incubation (Table 1). At 4 h, 45.5% of the original inoculum survived. Beyond 24 h, results were difficult to interpret because of variable growth of remaining fungi. Microscopic examination confirmed that clumping of organisms could not explain the apparent killing which was observed. Similarly, little or no phagocytosis of organisms was noted, as expected in a system nearly free of serum. But the high ratio of leukocytes to fungi in this system allowed the possibility that a small amount of phagocytosis might be missed, yet still result in significant killing. Therefore, incubations were performed with 10 μg ml^{-1} cytochalasin B, which completely inhibited phagocytosis of cryptococci. Others have noted that this concentration does not block antibody-dependent cell-mediated cytotoxicity[8], and only partially blocks cytotoxicity by immune spleen cells if cells are mixed to facilitate contact[9]. In three paired experiments, killing as reflected by percentage survival of the original inoculum was not significantly changed at 24 h in incubations containing cytochalasin B (mean ± s.e. of the mean survival 5.3 ± 5.3%, changed to 9.2 ± 6.9% with cytochalasin B).

Further dilution of antibody to 1 : 20,000 did not reduce killing (6.6% survival), but at 1 : 200,000 dilution, 73.3% of the original inoculum survived at 24 h. The optimum ratio was 50–100 leukocytes per cryptococcus. At a 20 : 1 ratio, 72.8% of the original inoculum survived, increasing to 88.0% at a 5 : 1 ratio. No killing was observed at 1 : 1 ratio (100% survival).

Preliminary studies indicated that human antibody can act in this system. Serum obtained from a patient with cryptococcosis was found to contain anti-cryptococcal antibody by an indirect fluorescent antibody technique[10]. When incubated in a 10% final concentration with mononuclear cells from two normal donors plus 10 μg ml^{-1} cytochalasin B to block phagocytosis, only 7.3% and 28.1% of the original cryptococcal inoculum survived at 24 h. In another experiment, normal serum was used which had no detectable anti-cryptococcal antibody by the indirect fluorescent antibody technique, but did have antibody present by a more sensitive absorption technique[11]. The IgG fraction from this serum was separated by the DEAE batch technique of Stanworth[12]. When 11 mg ml^{-1} was added to incubations, only 29.2% of the original cryptococcal inoculum survived at 24 h.

Table 1 Effect of Anti-cryptococcal Antibody on Survival of Cryptococci cultured with and without Mononuclear Cells

Leukocytes present*	Antibody present†	Survival of original cryptococcal inoculum (% at 24 h‡)
Yes	Yes	(6.8 ± 3.2)
Yes	No	(111.3 ± 7.6)
No	Yes	(158.0 ± 43.0)
No	No	(186.8 ± 49.0)

* 50–100 leukocytes : 1 cryptococcus.

† 1 : 2,000 dilution of hyperimmune serum or serum from the same rabbit obtained before development of antibody.

‡ Mean ± s.e. of the mean of experiments using leukocytes from nine donors. Original inoculum represented as 100%.

Triplicate 35 mm plastic dishes each contained approximately 5×10^4 cryptococci quantified by plate counts, with or without varying ratios of leukocytes in 1 ml of medium. Media consisted of heated (56° C for 30 min) rabbit serum diluted 1 : 2,000 or greater with Eagle's minimal essential medium enriched as outlined elsewhere[20]. Dishes were incubated at 37° C in 5% CO_2 with continuous mixing on a rocker platform for 24 h. Leukocytes were lysed and clumps dispersed using Triton X-100 in saline (1 : 1,000 final dilution when added to culture dishes). Dishes were scraped with a rubber policeman and washed four times with Triton X-100 into a test tube which was vigorously mixed. This treatment did not decrease viability of cryptococci. Further tube dilutions were performed in distilled water. Live organisms were quantified by plate counts and the number compared with the original inoculum to measure any growth or killing which occurred during the period of incubation.

Separated mononuclear cells suspended in 10 ml of medium were incubated in 100 × 20 ml plastic dishes on rocking platforms at 37° C for 30 min in 5% CO_2. Non-adherent cells were removed and the procedure was repeated twice. When these non-adherent cells were used in incubations, killing was reduced 60% compared with incubations where adherent cells were not removed. This suggests that the killer cells in this system have some capacity to adhere to surfaces. If this system is analogous to antibody-dependent cell-mediated lysis of erythrocytes or other target cells as described by several workers[13–17], my results are consistent with those reported by Van Boxel using mouse spleen effector cells[17]. Studies are in progress to identify the specific effector cell which can kill cryptococci. Because of the heterogeneous nature of the mononuclear cell population used in this system, the possible roles of monocyte-like cells[18], B lymphocytes[17], or other cells is still uncertain.

In any case, it seems that mononuclear cells in the presence of specific antibody can kill a microorganism, *C. neoformans*, by a non-phagocytic mechanism. The relevance of these observations to *in vivo* conditions remains to be established. It may, however, be noteworthy that the presence of anti-cryptococcal antibody in the serum of patients with cryptococcosis has been correlated with eventual cure[10]. Antibody-dependent cell-mediated killing as well as other cellular non-phagocytic killing mechanisms[19] may well be important in dealing with microorganisms which are not readily ingested by phagocytic cells.

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Mechanism of Action of Dental Electro-anaesthesia

NEWMAN[1] has proposed that the analgesia produced by passing a small electric current from the dental drill (positive) to a hand electrode (negative) is due to anodal blocking of nerve fibres within the tooth. Fields *et al.*[2] feel that the likely mechanism is electrocoagulation of odontoblasts in the dentinal tubules under the site of drilling. The difference is clinically important, for anodal blocking is a harmless reversible process whereas the destruction of odontoblasts leaves permanent dead tracts in the dentin under the site of application of the current. These two mechanisms can be separated by applying stimulation and electro-analgesic current to different sites on the same tooth, since the local mechanism (electrocoagulation) would have no effect in blocking a stimulus applied elsewhere.

Ten cats were prepared with chronic amalgam implants on the upper canine teeth[3,4]. Eight of these had two-electrode implants; two were prepared with three-electrode implants. The stimulating, anaesthetising, and recording arrangements were as shown in Fig. 1. Current was applied from an implant close to the apex of the pulp chamber, with a return lead from a stainless steel screw embedded in the skull, simulating normal dental electro-analgesic practice. Test stimuli were applied from another tooth implant to the skull (Fig. 1*A*), or between two other tooth implants (Fig. 1*B*). The jaw opening reflex[3,4], monitored electromyographically, was used as the index of pulp excitation. The cats were tested repeatedly at intervals over a period of 3 to 4 months. All were awake; the test stimulus used does not induce overt distress or escape behaviour in the cat provided the pulse is brief, mild and not too frequent[4,5].

Typical results are shown in Fig. 2. Anodal current reduced or blocked the reflex response; cathodal current increased it. The smallest effective current was 20 μA, and typically 40 to 60 μA was required for a full block. The blocking tended to be cumulative, in the sense that successive blocks required less current. There was, however, complete recovery between sessions.

In a few trials, much larger currents (500 to 2,000 μA) were used for comparison with the practice of ionic desensitisation[6,7], which is known to produce local damage to odontoblasts. On cessation of this blocking current, the cats tested were visibly distressed and drank copiously; there were no obvious permanent after effects.

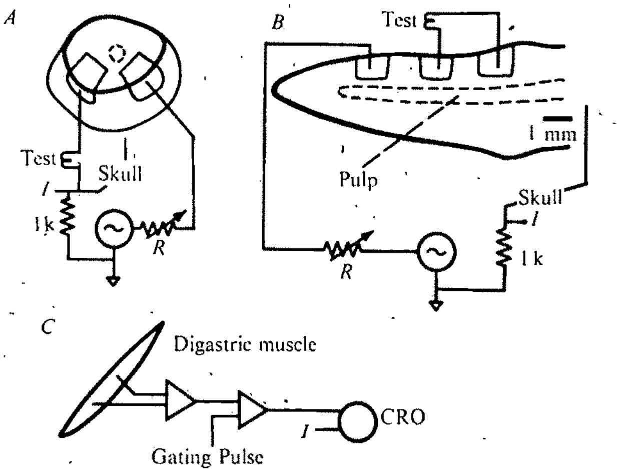

Fig. 1 Three-terminal (*A*, eight cats) and four-terminal (*B*, two cats) arrangements used for testing anodal blocking of pulp nerves. The test pulse was 0.2 ms long, 200 to 500 μA in amplitude, derived from an r.f.-isolated bipolar source which had an internal resistance of 100 kΩ. In arrangement *A* the polarising and test currents both passed through the tooth root to the skull; in *B* the test pulse was confined within the tooth. Point *I* was connected to the lower beam of the oscilloscope to record current passed through the polarising electrode. The signal generator was operated at constant voltage (centre zero, range ±10 V) and the polarising current was varied by changing *R* within the range 0.1 to 1.0 MΩ. The digastric EMG was monitored as shown in *C*. Hook electrodes, of platinum or nichrome wire, 1 cm long and 0.25 mm diameter, were chronically implanted about 1 cm apart in each digastric muscle. Leads from a skull plug were run to a differential preamplifier of gain 100, bandwidth 80 Hz to 10 KHz, input impedance 10 MΩ. The output from this amplifier went to a gated amplifier which displayed, on the upper beam of the oscilloscope, the segment of EMG waveform occurring 4 to 13 ms after each test stimulus (see Fig. 2).

The currents found adequate to block the jaw opening reflex in this study are comparable in magnitude with those effective in clinical electro-analgesia[1,2,8]. The site of testing is separated from the site of injection of the polarising current, so the block of response cannot be due to an inactivation of receptors in the dentin under the polarising electrode. The reversible blocking seen is consistent with the known properties of anodal blocks on other nerves[9]. Thus, it seems that anodal polarisation of pulp nerves may be a significant mechanism in the clinical relief of tooth pain. These findings do not exclude the possibility that local damage to odontoblasts may also play a role. [illegible]ars of clinically significant damage produced by curre[illegible] of 60 μA or less are, however, probably unwarranted, since much larger currents have been used routinely for dentin

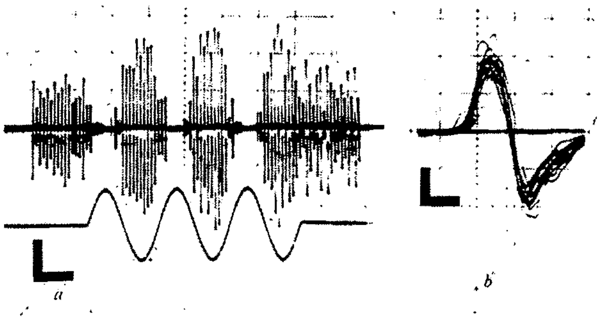

Fig. 2 *a*, Variation of digastric EMG amplitude with polarising current. Each vertical line on the upper trace is a 9 ms segment beginning 4 ms after a test stimulus to the tooth. Test stimuli are applied at a rate of 2 s^{-1}. Calibration: upper trace, 2 mV; lower trace, 20 mV (equivalent to 20 μA); time, 5 s. Lower trace shows the polarising current applied; tooth positive (anodal) is up. *b*, Detail of EMG waveform. Ten sweeps with stimulus, five without. Calibration: 2 mV; time, 2 ms. Only the gated portion of the response is shown.

desensitisation for many years[6,7].

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Variation with Sex of Irradiation-induced Chromosome Damage in Somatic Cells of *Drosophila melanogaster*

IN *Drosophila melanogaster*, much information has been collected on chromosome mutations analysed by genetic methods, but there has so far been no cytological analysis of the chromosome mutations induced in somatic cells. By irradiating cells of *D. melanogaster*, in which we knew that somatic pairing was present, we have also wished to extend our previous studies[1], carried out on endoreduplicated Chinese hamster cells, to verify the influence of chromosome pairing on the type and frequency of induced chromosome aberrations.

Third instar larvae of two stocks (Oregon R and *b cn vg bw*) were irradiated with 312.5, 625 and 1,250 R of X rays (180 kV, 6 mA, 3 mm Al and 125 R min^{-1}). At various times after irradiation, samples were prepared by squashing larval ganglia in acetic orcein after fixation for 3 min in a 1 : 1 mixture of methyl alcohol and acetic acid. Before fixation, the ganglia were kept for 2 h in a 0.7% aqueous solution of NaCl containing 10^{-4} M colchicine.

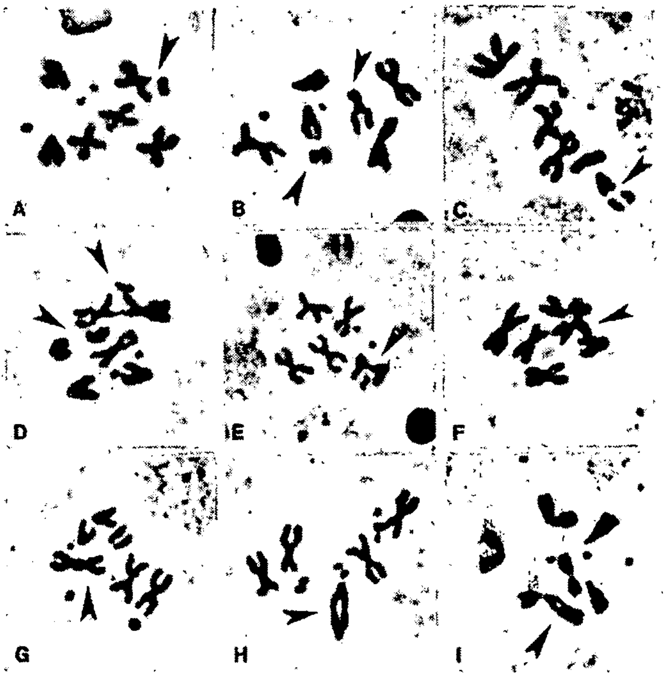

Fig. 1 Induced chromatid and chromosome aberrations in larval ganglion cells of *D. melanogaster*. A, (♀) Terminal deletion of an autosome; B, (♀) isochromatid deletion of an autosome; C, (♂) isochromatid deletion of an X chromosome; D, (♀) asymmetrical exchange between autosomes plus isochromatid deletion of an autosome; E, (♀) asymmetrical exchange between X chromosomes; F, (♀) symmetrical exchange between autosome and X chromosome; G, (♀) dicentric between autosomes with associated fragment; H, (♀) dicentric between X chromosomes with associated fragments; I, (♀) translocation between autosomes.

Figure 1 shows the main types of chromosome and chromatid aberration that we could score in this material. The genome of *D. melanogaster* is suited to this type of analysis, as the

Table 1 Frequencies of Chromatid and Chromosome Aberrations in Larval Ganglion Cells of *D. melanogaster* after Treatment with X Rays

Stock	Dose (R)	Fixation time post-irradiation (h)	Sex	No. of cells with aberrations	Chromatid deletions	Isochromatid deletions and chromosome deletions †	Chromatid exchanges	Dicentrics	Scorable translocations and inversions	Chromatid gaps	Isochromatid gaps
Oregon R	0 (Control)	—	♀♀	5	1	4	—	—	—	—	2
			♂♂	2	2	—	—	—	—	1	—
	312.5	4	♀♀	178	18	181	7	—	—	6	4
			♂♂	123*	13	111*	2	—	—	5	3
	625.0	4	♀♀	316	39	326	20	1	—	6	8
			♂♂	225*	32	205*	10	3	—	5	9
	1,250.0	2	♀♀	540	219	338	130	—	—	31	17
			♂♂	448*	148*	306	72*	—	—	28	12
		4	♀♀	505	115	477	54	16	—	16	18
			♂♂	362*	72*	357*	16*	8	—	11	10
		8	♀♀	436	19	394	4	125	30	5	4
			♂♂	314*	25	261*	3	77*	12*	3	4
		10	♀♀	435	10	296	—	238	36	4	2
			♂♂	294*	6	233*	3	110*	22	3	2
b cn vg bw	625.0	4	♀♀	409	94	376	17	10	1	13	11
			♂♂	321*	60*	285*	9	8	—	14	10

* Significant in χ^2 test with $P < 0.01$.

† An isochromatid deletion can result both from the replication of a chromosome breakage (chromosome deletion) and from an isochromatid breakage. Consequently this type of aberration is present at both early and late fixation times post-irradiation.

1,000 metaphases of at least ten larvae scored for each point.

aberrations are clear and the number of chromosomes per cell is small and two pairs are easily distinguished from the others. In the colchicinised metaphases, the somatic pairing is lost; however, the pairing is clearly present up to late prophase.

Table 1 shows the data obtained in one series of experiments. The experiments were carried out mainly on the Oregon stock and then on the *b cn vg bw* stock for confirmation. The larvae of the Oregon stock irradiated with 1,250 R were killed at various times after irradiation to obtain data on the different stages of the cell cycle: in this way it was possible to study chromatid and chromosome aberrations.

The main result that emerges from the data of Table 1 is that, in the females of both the stocks at all three doses of irradiation, there is a greater frequency of chromosome or chromatid aberrations of every type.

In the present state of our studies it is difficult to determine the cause of this difference: a difference in the efficiency of repair both of premutational damage and of the broken chromosomes might be involved[2,3]. In this respect it is natural to think of the possible presence in the females, as opposed to the males, of the enzymes involved in crossing-over, and of the possibility that, for the formation of a chromosome aberration, some kind of rectification of lesions in DNA is necessary[4,5].

Another fact worth emphasising is the almost total absence in our results of intra-exchanges, either at chromatid or at chromosome level. We maintain that this is one of the effects associated with the chromosome pairing.

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Radiochemical Purification of Bacteriophage λ Integrase

BACTERIOPHAGE λ has evolved a highly specific mechanism by which it integrates its DNA into the *E. coli* genome. The product of the lambda gene *int*, which I call integrase, mediates the insertion of the phage DNA at specific attachment sites on both the phage and host genomes[1–3]. A non-enzymatic method by which integrase can be identified in infected cells has been described previously[4,5]. It depends on the observation that in cells which have been heavily irradiated with ultraviolet light before infection with phage λ, synthesis of host proteins and most phage proteins is markedly depressed[6]. Under these conditions a high percentage of labelled amino acids is incorporated into phage-specific proteins including integrase. Using a differential labelling technique, integrase is identified by means of sodium dodecyl sulphate (SDS) gel electrophoresis as the only protein species eliminated by a nonsense (chain-terminating) mutation in the *int* gene.

I describe here the purification of integrase, monitored by electrophoretic assay, to radiochemical homogeneity. In an independent study, Nash[7] has used a similar method to obtain radiochemically pure integrase, which he calls Int protein.

Figure 1*a* illustrates electrophoretic identification of labelled proteins synthesised in a heavily irradiated host by either *int*$^+$ phage or *intam* (*int*29) phage. One major peak at molecular weight 44,000 is synthesised only by the *int*$^+$ phage and is thus concluded to be integrase.

The interpretation that the peak at 44,000 molecular weight is integrase is corroborated by the following experiments. (1) I isolated two additional *int* amber mutants, *int*405 and *int*920, and these mutations also eliminate the peak at molecular weight 44,000. *Int*405 is different from *int*29 and *int*920 since it is suppressed only by $su_{\rm I}^+$ hosts whereas *int*29 and *int*920 are both suppressed by $su_{\rm II}^+$ hosts. (2) The protein peak at molecular weight 44,000 is found if the infection with *intam* phages is carried out in a *su*$^+$ host. (3) The *bio*16-A deletion spans a region of DNA, from the attachment site to within the *int* gene[8], which would code for a polypeptide of molecular weight approximately 22,500 (ref. 9). I have shown that the *bio*16-A deletion eliminates the peak at molecular weight 44,000, thus ruling out the possibility that this peak is coded by a gene adjacent to *int* but is eliminated by *int* amber mutations because of polarity (4) The peak at molecular weight 44,000 is not affected by the non-suppressible mutation *int*6 (ref. 10) or by the temperature-sensitive mutation *int*1 (ref. 11). This experiment indicates that this peak contains the protein defined by the *int* amber mutations rather than a protein under *int* control or a protein controlling integrase production.

We have shown previously that in crude extracts integrase is associated with large, fast-sedimenting particles[5]. Treatment at high ionic strength dissociates integrase from these particles and leaves it in a slower sedimenting form. This observation forms the basis for a purification procedure. Large integrase particles are collected by centrifugation in low salt and then resedimented in 0.66 M KCl. The integrase is then concentrated by ammonium sulphate precipitation and finally fractionated by Sephadex G-100 chromatography. During the purification integrase is monitored solely on the basis of its mobility on SDS gel electrophoresis (see legend to Fig. 1 for details). Gel electrophoresis of radiochemically pure integrase is shown in Fig. 1*b*. The radiochemical purification of integrase represents approximately a 100-fold physical purification. However, the most purified fractions still contain a 100 to 1,000-fold excess of unlabelled proteins.

Depending on its state of purification and on the ionic strength of the buffer, integrase exists in one of three forms, characterised in part by markedly different sedimentation rates. Following sonication, integrase is found in a heterodisperse collection of large (>100S) particles. These particles contain DNA; treatment with pancreatic DNase disperses the large particles into species of 35S or smaller. After purification to homogeneity, all of the integrase sediments in low ionic strength (<0.1 M KCl) as a fairly homogeneous peak at 35S with a broad tail at low molecular weights. It is unlikely that the 35S form is an integrase-DNA complex since treatment of the 35S form with pancreatic DNase has no effect on its sedimentation properties.

Both the large integrase particles found in crude extracts and the 35S aggregate are disrupted by high ionic strength buffers. In 0.66 M KCl, regardless of its stage of purification, integrase sediments as a homogeneous peak at $S_{20,w}$ approximately 4.0. A sedimentation rate of 4S corresponds to a globular protein of molecular weight 60,000. Since the monomer molecular weight of integrase is 44,000 as determined by SDS gel electrophoresis, the 4S form could either be a monomer or a dimer.

Formation of the 35S aggregate is reversible: high salt (0.3 M KCl) disrupts the aggregate, but the aggregate is reformed on return to low ionic strength. This indicates that 35S aggregate formation is an intrinsic property of integrase which has not been lost due to denaturation.

Since integrase catalyses a site-specific recombination event *in vivo*, radiochemically purified integrase might inter-

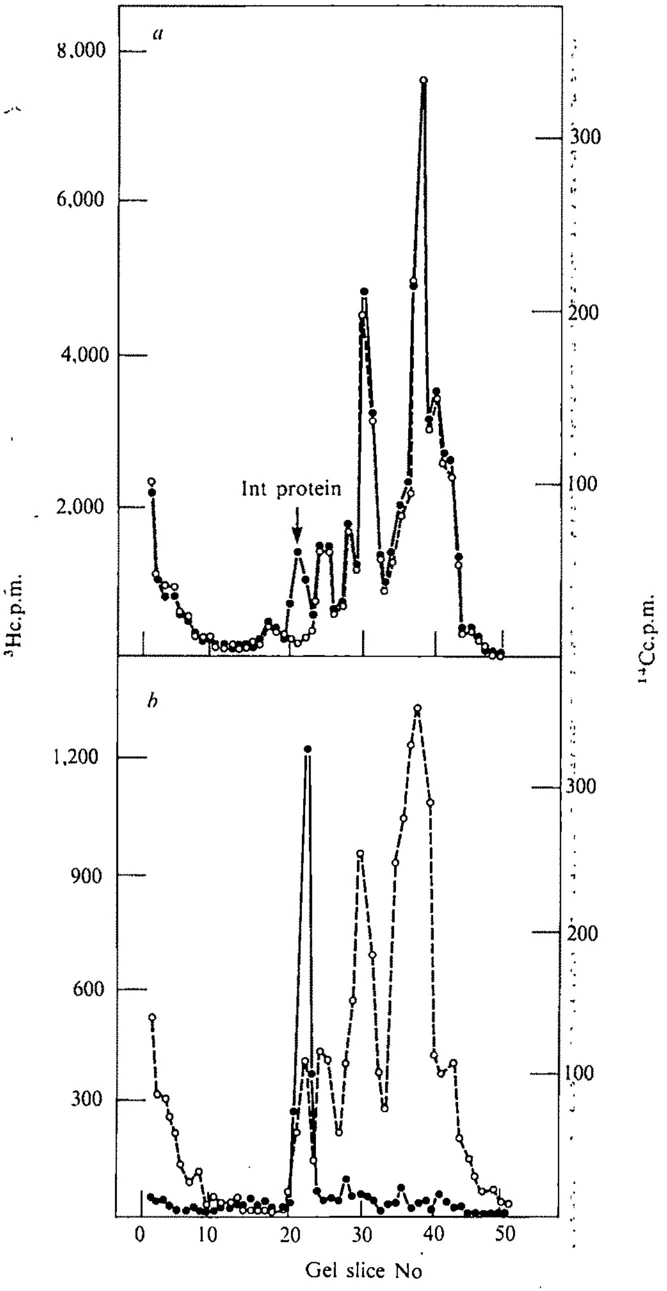

Fig. 1 Electrophoretic identification and radiochemical purification of integrase. *Escherichia coli* strain 159 (*su$^-$gal$^-$str-r uvr$^-$*) was grown at 37° C to a density of 3×10^8 ml^{-1} in I medium (0.37% Na$_2$HPO$_4$, 0.3% KH$_2$PO$_4$, 0.25% NaCl, 0.00075% FeCl$_3$, 0.4% maltose, 0.001 M MgSO$_4$). The culture was chilled and then irradiated in 120 ml aliquots in an 18 × 8 inch dish with two 15 watt GE germicidal bulbs at a dose of 8,400 ergs mm^{-2}. Ten 100 ml aliquots of the irradiated culture were added to ten prewarmed flasks containing 1 mCi ^{3}H-leucine (50 Ci mmol^{-1}) and 2 ml 1.0 M MgSO$_4$, and infected with λ*Eam4b2red*β*am270cI857Sam7* at a multiplicity of five particles per cell. Two further 100 ml aliquots of irradiated cells were treated in the same way, except that one was labelled with 25 μCi ^{14}C-leucine (250 mCi mmol^{-1}) instead of ^{3}H-leucine and the other was infected with λ*Eam4b2intam29red*β*am270cI857Sam7* and labelled with 25 μCi ^{14}C-leucine (250 mCi mmol^{-1}). After incubation for 1 h at 37° C, the combined ^{3}H-labelled cultures and the two ^{14}C-labelled cultures were washed repeatedly with cold I medium and each resuspended in 5 ml TMES buffer (0.01 M Tris, *p*H 7.5, 0.001 M MgCl$_2$, 0.0002 M EDTA, 0.1 mg ml^{-1} dithiothreitol). Each culture was sonicated with cooling for 1.5 min at 60 W and then centrifuged at 10,000 r.p.m. for 10 min to remove debris. The ^{3}H-labelled lysate was further purified by sedimentation into a 2 ml 50% glycerol cushion in TMES buffer at 4° C for 3 h at 40,000 r.p.m. in an IEC SB283 rotor. The glycerol cushion was brought to 0.67 M KCl and 10% glycerol and then spun at 4° C for 12 h at 25,000 r.p.m. in an IEC SB110 rotor onto a 23 ml 40% glycerol cushion containing 0.67 M KCl and TMES buffer. The supernatant fluid above the cushion was collected and brought to 75% saturation with cold, neutralised, saturated ammonium sulphate. After incubation overnight at 4° C, the precipitate was collected by centrifugation at 4° C for 30 min at 18,000 r.p.m., resuspended in 2 ml TMES buffer supplemented with 50 μg ml^{-1} bovine serum albumen (BSA) and 10% ethylene glycol, applied to a 1.5 × 30 cm Sephadex G-100 column equilibrated with the resuspension buffer, and then washed through with the same buffer at 4° C at a flow rate of 10 ml h^{-1}. The fractions containing material excluded from the column were pooled and found to contain 60–80% of the integrase applied to the column. The yield from crude extract was 35%. Integrase was monitored during purification solely on the basis of its mobility on sodium dodecyl sulphate (SDS) gel electrophoresis. This was made possible by the construction of special phages containing a combination of amber mutations (*Eam4* and *red*β*am270*) and a deletion (*b2*) such that they synthesised no other proteins with an electrophoretic mobility near that of integrase. To ensure the correct identification of integrase during the purification, each SDS gel of a ^{3}H-labelled fraction was internally calibrated with unfractionated ^{14}C-labelled *int*$^+$ or *intam* lysate. In preparation for gel electrophoresis equal aliquots of ^{3}H-labelled fractions and unfractionated ^{14}C-labelled lysate were combined and incubated at 90° C for 5 min with 0.2% SDS and 0.1% dithiothreitol. A 0.05 ml sample of this mixture was subjected to electrophoresis on a 5 × 0.6 cm 10% acrylamide gel. *a*, Unfractionated ^{3}H-labelled *int*$^+$ lysate (●) plus unfractionated ^{14}C-labelled *int* amber lysate (○); *b*, purified ^{3}H-labelled integrase (pooled G-100 fraction) (●) plus unfractionated ^{14}C-labelled *int*$^+$ lysate (○). Electrophoresis is from left to right.

act specifically with DNA which contains the phage attachment site. To test the possibility of specific *in vitro* binding, radiochemically pure integrase was cosedimented with DNA in glycerol gradients or incubated with DNA and then filtered through nitrocellulose filters.

Integrase in the 4S form does not bind to DNA either specifically or non-specifically, probably due to the relatively high salt concentration (>0.3 M KCl) necessary to maintain the 4S state. On the other hand, when 35S integrase aggregates are mixed with a large molar excess (500–2,500-fold) of 32S λ DNA, the integrase sediments with the DNA at approximately 32S. The same result is obtained with both wild type DNA and with DNA which has the attachment site deleted (*att*∇). These results suggest that 35S integrase binds non-specifically to λ DNA, and further, that this binding disrupts the 35S aggregate. This interpretation was tested by the following experiment. Integrase aggregates (35S) were cosedimented with a mixture of whole *att*$^+$ DNA and sonicated *att*∇ DNA (and *vice versa*). If the 35S aggregates disperse when binding non-specifically to DNA, then a significant amount of integrase should sediment with the sonicated *att*∇ (or sonicated *att*$^+$ DNA) at 5S. The result of this experiment is shown in Fig. 2: after sedimentation, the integrase partitioned almost equally between the *att*$^+$ and *att*∇ DNAs. As above these results give no indication of specific binding and, in addition, make it seem unlikely that a relatively weak non-specific binding is obscuring a much stronger specific binding to the attachment region. There was no increase in binding specificity over a wide range of salt concentrations and in various buffer systems containing different cations.

In addition to binding to λ DNA in glycerol sedimentation gradients, radiochemically pure 35S integrase aggregates bind λ DNA to nitrocellulose filters. The filter-binding activity in the preparations was shown to be due specifically to integrase since material from an *int* amber infection, carried through the same stages of purification as the *int*$^+$ lysate, did not display any filter-binding activity. Integrase displayed the same amount of filter-binding activity with both *att*$^+$ and *att*∇ DNA.

The experiments described here demonstrate that partially purified integrase binds non-specifically to λ DNA and

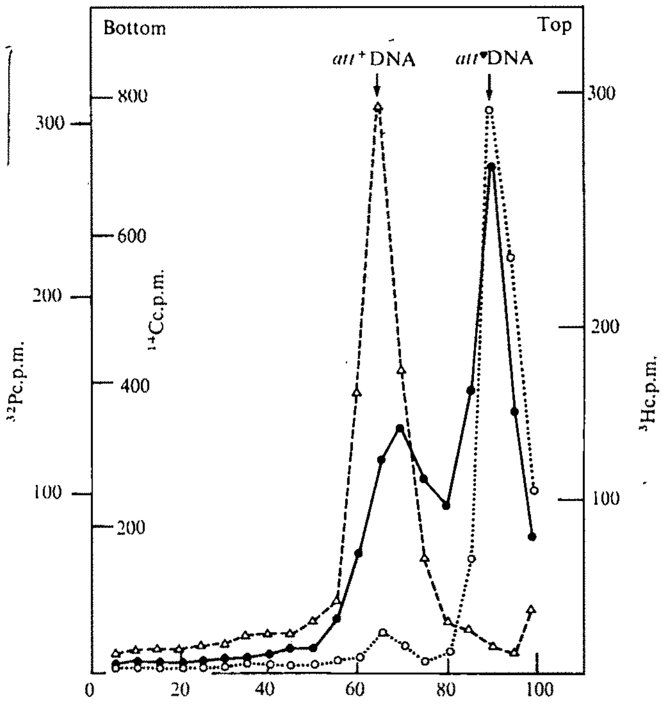

Fig. 2 Cosedimentation of 35S integrase with whole *att*$^+$ and sonicated *att*▽ λ DNA. A 50 μg ml^{-1} solution of ^{14}C-labelled λ*b104cI857Sam7* DNA (*att*▽) was sonicated with cooling for 2 min at 60 W in 0.01 M Tris, *p*H 7.5, and 0.001 M EDTA. A 25 μl aliquot of radiochemically purified integrase in the 35S form (pooled G-100 fractions from Fig. 1) was incubated at 4° C for 1 h with a mixture of 5 μg whole ^{32}P-labelled λ*cI857Sam7* DNA and 5 μg sonicated ^{14}C-labelled λ*b104cI857-Sam7* DNA. The incubation mixture contained 0.01 M Tris, 0.001 M MgCl$_2$, 50 μg ml^{-1} BSA, and 10% ethylene glycol. The DNA-integrase mixture was layered on 3.6 ml 10–30% linear glycerol gradient containing the same ingredients as the incubation buffer, and then spun at 4° C for 2 h at 60,000 r.p.m. in an IEC SB405 rotor. The gradient was dripped directly into scintillation vials and counted. When the experiment was repeated with sonicated *att*$^+$ DNA and whole *att*▽ DNA, essentially the same result was obtained, that is, integrase counts partitioned approximately equally between the whole and sonicated DNAs. When integrase was cosedimented only with sonicated *att*$^+$ or *att*▽ DNA all of the integrase sedimented with the sonicated DNAs. ^{3}H-labelled integrase (●); ^{32}P-labelled λ*cI857Sam7*(*att*$^+$) DNA (△); ^{14}C-labelled sonicated λ*b104cI-857Sam7* (*att*▽) DNA (○).

reversibly forms a fairly homogeneous aggregate which sediments at approximately 35S. With further purification of integrase it may be possible to demonstrate the existence of an integrase-mediated reaction specific for attachment region DNA and to elucidate the role of the 35S aggregate in this process. One can speculate, for example, that the 35S aggregate functions as a structural cage to hold pairs of attachment sites together.

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Changes in Rates of Increase in Size and of Exoskeletal Production during Old Age in the Isopod *Ligia oceanica* (L.)

THE male isopod *Ligia oceanica* (L.) goes on moulting and growing even when its spermatogenesis has come to an end[1–4]. The coexistence of growth and senescence seems to me an unexpected finding in a higher metazoon. It is possible to split up the postpuberal growth period of *Ligia* into the sexually mature stage and the senescent stage, both of which are states of morpho-physiological equilibrium, and an intermediate transitional phase. The term 'stage' is here used as equivalent to the French term *étape* and is composed of several 'stadia' separated by a moult. In the sexually mature stage the gonads are fully developed, in the senescent stage they are degenerated. The transitional phase is characterised by various degrees of testicular degeneration.

Sexual deterioration is not, of course, the only criterion of senescence, and I have shown[5] that the respiratory rate falls off strongly during the senescent stage. In another study[6], weight and calcium content of the exuvium were found to vary more during the senescent stage than during the sexually mature stage. I thought that loss of vitality would thus lead to greater metabolic irregularity of exoskeletal formation and of exuviation and calcification, and wondered whether the senescent animal is different from the younger adult in other quantitative exoskeletal characters than merely greater exuvial variation. I have therefore undertaken a comparative study on the growth rate during the two stages and the transitional phase.

Isopods were collected during the spring tides on the coast of Normandy (Luc-sur-Mer, Calvados, France, in May 1966 and July 1967). Some 200 premoulting adult males of various sizes were then reared in the laboratory at a temperature of 17° C, for two successive moults. Most survived

Table 1 Rate of Increase of Head Breadth

Stage or phase	Number of individuals	$\bar{R}$	σ
Sexually mature	27	1.0702	0.00652
Transitional	30	1.0471	0.00691
Senescent	27	1.0155	0.00449

The rate R is defined by $R = H_1^{-1}H_2$, where H_1 and H_2 are the head breadth at two consecutive stadia. $\bar{R}$, geometrical mean of R; σ, standard deviation of log R.

a first moult but only eighty-four survived the second. The head breadth and the dry weight of the exuvium of each individual were measured.

The growth rate from one stadium to the next generally varies with the environment, and in crustacea seems to be lower in the laboratory than in the wild. In the premoult state where the animals were caught, the conditions that determine the characteristics of the moult were, however, already laid down. A comparison of the exuviae of first and second moult, which show sizes of animals at the two successive stadia before and after capture, may therefore give a fair picture of growth rate in the wild.

The treated animals were divided into three groups corresponding to the two stages and the transitional phase. The geometrical mean of the growth rate was calculated for each group (Table 1). The growth rate was markedly lower in the senescent stage than in the sexually mature stage; the difference (reckoned by a t-test carried out on the logarithm of the growth state) is highly significant ($P<0.001$).

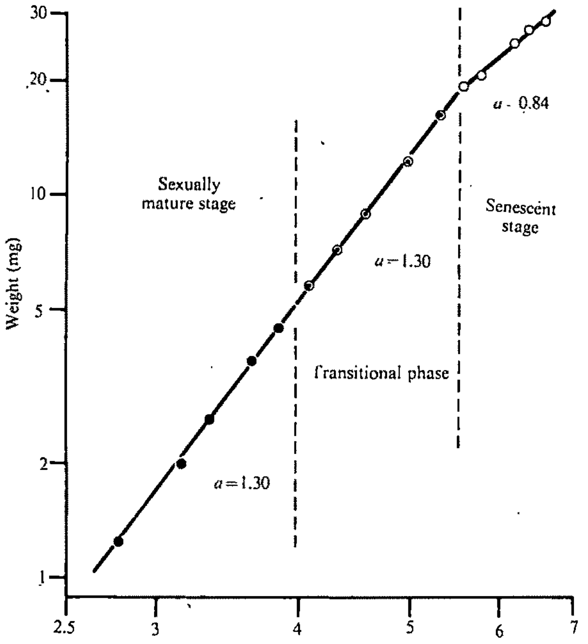

Fig. 1 Relative increase of weight (W) to head breadth (H) of the exuvium. Each point stands for the geometrical mean of ten individuals. a, Regression coefficient of log W on 3 log H.

I then studied the relation of weight (W) to head breadth (H) of the exuvium in 150 individuals, to estimate the rate of exoskeletal production at each stadium. This should give an indication of anabolic intensity and thus of growth capacity in mass. The individuals belonging to each stage or phase were grouped by tens according to their H value. The geometrical means of H and W for each such class were plotted on a double logarithmic grid (Fig. 1). The figure displays a similar growth curve for the sexually mature stage and for the transitional phase, but the curve for the senescent stage is different, with a lower regression coefficient a of log W on 3 log H. Reeve's stochastic test[7] shows this difference of a to be highly significant ($P<0.001$). The growth rate in mass may be deduced, from this lower value of a, to fall off with old age. Thus, although an aged isopod can moult and grow, notwithstanding testicular degeneration, its senescence is fairly marked by a fall in the rates of increase of size and mass.

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Lantern Fish compare Downwelling Light and Bioluminescence

THE biological utility of the light organs of oceanic fishes has been the subject of much speculation[1,2]. According to one hypothesis the ventral photophores of some mesopelagic fishes in the upper regions of the sea may produce bioluminescence that matches the colour and intensity of environmental light passing downwards on either side of the fish. Such a system of camouflage could obscure the ventral outline of the fish when viewed from below[3,4].

This hypothesis is supported by several lines of evidence. First, light in the sea is highly directional, the ratio of upward to downward directed light being small (1:200 to 1:100) in clear oceanic waters. Consequently, the reflecting dorsal and lateral surfaces of a fish enable it to match the background when viewed from above and from the side, but the ventral silhouette seen from below would appear dark against the brighter background[5]. Second, the blue wavelengths of the spectrum penetrate most deeply into the ocean, and therefore daytime illumination at depths of 500 to 1,000 m is blue and dim. Bioluminescence of some midwater fishes is blue and falls within the range of intensities measured during the day at these depths and has a very similar angular distribution[6–9].

One species of midwater fish has proved invaluable in the study of this hypothesis and its implications. *Tarletonbeania crenularis* is a common lantern fish (Myctophidae, Iniomi) living in the North Pacific Ocean in deep water beyond the continental shelf. Unlike many other mesopelagic fish, this species migrates to the surface at night from daytime depths of 500 to 1,000 m and is often associated with sonic scattering layers[10–12]. Lantern fishes are bioluminescent. Light organs or photophores occur in rows along the ventral surface of the head and body and groups of isolated organs may be found around the eyes. Each dermal photophore consists of a mass of innervated photogenic tissue backed by a pigmented mantle and reflector. Some light organs are covered by a superficial lens, scale and reflector system which concentrates and collimates the light from the photogenic tissue and modifies the colour and intensity of the emitted light[13–15].

If the ventral surface of a lantern fish is to remain invisible in the face of changing intensities of incident light, the fish should be able to monitor both the environmental illumination and the activity of its photophores. *Tarletonbeania crenularis* may see downwelling light and bioluminescence from a supraorbital photophore, and adjust the output of its ventral photophores to match environmental illumination.

Lantern fishes swimming near the surface on calm, dark nights are attracted to an underwater light and can be collected with dipnets and maintained in fairly good condition in darkened aerated aquaria for several hours. Fishes kept in total darkness rarely luminesced, but when they were illuminated from above with blue light the ventral photophores of some specimens emitted a bluish light lasting for about 15 min which approximated the colour and intensity of the incident illumination and which was extinguished when the incident illumination was turned off. The bioluminescence was only visible after about 30 min of dark adaptation on the part of the observer when the intensity of the incident light was very dim. It was not possible to determine whether the supraorbital photophore was luminescing simultaneously with the ventral photophores as the bioluminescence very closely resembled the colour and intensity of the incident light, and the relationships of the orbital photophores to the eyes of *Tarletonbeania* were studied for evidence of a feedback loop between the bioluminescent and visual systems.

The eyes of lantern fishes are large and well adapted for vision in dim light. The nearly spherical lenses of a 41 mm ♀ *Tarletonbeania* were 1.3 mm in diameter and protruded well through the pupils; the centres of the lenses were nearly in line with the iridial margins and coincident with the centres of the pupils (Fig. 1). The protruding lenses of fishes (refractive index 1.67) compensate for the absence of corneal refraction, since corneal tissues have the same refractive index (1.33) as water[14].

The eye of *Tarletonbeania* is particularly distinguished by the presence of a crescent shaped gap or aphakic aperture between the central margin of the iris and the ventral border of the lens. This aperture has not been described in lantern fishes before but is found in other bathypelagic species[15]. The aphakic aperture of *Tarletonbeania crenularis* is so placed that light rays from the supraorbital photophores and from the dorsal visual fields can reach the retina (Fig. 2).

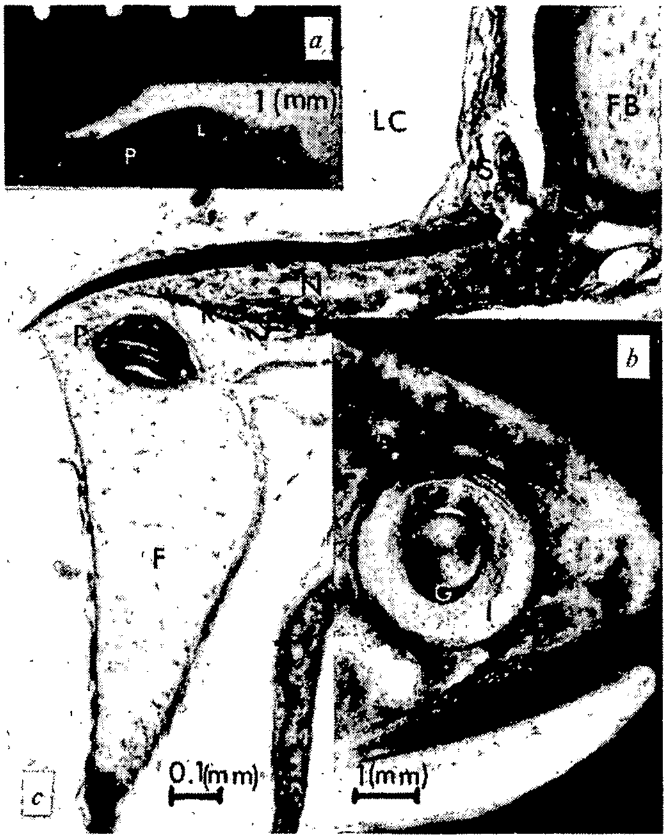

Fig. 1 Structure of the eye and supraorbital photophore of the lantern fish, *Tarletonbeania crenularis*. *a*, Dorsal view of the left eye of a 41 mm ♀ specimen, showing the protruding lens, L, subjacent to the cornea, and P, the position of the supraorbital photophore. *b*, 10 μm paraffin section stained with Mallory triple connective tissue stain; F, transparent fold of tissue in which photophore, P, is located; R, reflector; N, branch of supraorbital nerve; SN, innervating the photophore; FB, frontal bone; LC, supraorbital lateral line canal; C, cornea. *c*, A lateral view of eye of *Tarletonbeania* showing the supraorbital photophore; P, (the fold of tissue over photophore has been removed); L, lens; G, aphakic aperture between lens and the iridial margin; I, iris.

The supraorbital light organ (depicted in figures of Bolin[18], but not described) is situated in a flap of tissue at the posterodorsal margin of the orbit. This organ is innervated by a branch of the supraorbital nerve and is enveloped posteriorly and medially by a reflector and pigmented epithelium (Fig. 1*b*)[16].

Light from the supraorbital photophore falls on the posterior-superior surface of the lens and the anterior-inferior quadrant of the retina. The approximate paths of light rays from the supraorbital photophore and from the dorsal visual fields through the lens and onto the retina were constructed ignoring corneal refraction. The distances from the supraorbital photophore to the surface of lens, the diameter of the lens, and the size of the aphakic aperture were measured from photographs of dissected preparations and histological sections. An almost constant feature of fish eyes is the ratio of the distance from the centre of the lens to the retina divided by the radius of the lens. This ratio (Matthiessen's ratio) is about 2.55 and it was used to obtain the distance from the centre of the lens to the retina to compensate for errors in measured distances created by artefacts of fixation. Snell's law and a value of 1.67 for the refractive index of the lens were used to determine the angles of refraction at the surfaces of the lens[17-19]. The constructed path of light rays from the supraorbital photophore was confirmed by splitting the head of a fresh fish, dissecting the orbit away from the back of the retina, and directing a beam of light from a fibre optic system from the anterior retinal quadrant backwards through the aphakic aperture and lens and onto the supraorbital photophore.

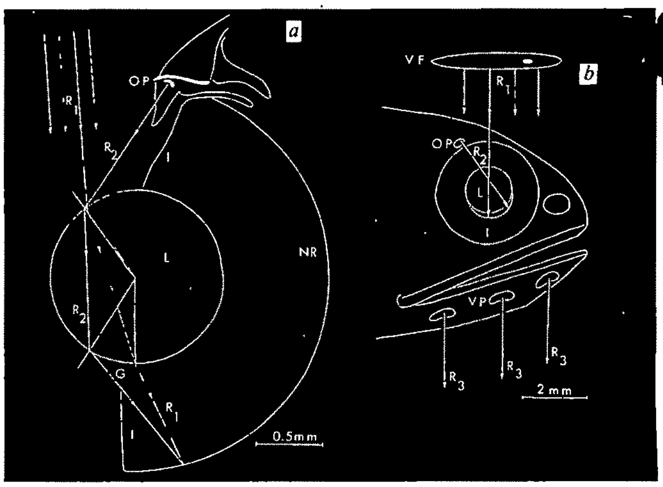

Fig. 2 Optical paths taken by light from the dorsal visual field and from the supraorbital photophore of *Tarletonbeania crenularis* through the dioptric media of the eye. *a*, Diffuse downwelling light from the dorsal visual field, R_1, and a ray of light, R_2, from the supraorbital photophore, are bent by the lens, L, and fall on the neural retina, NR. Some light passes through an aphakic aperture, G, between the iris, I, and the lens. *b*, A lateral view of light rays R_1 from the dorsal visual field, VF, and R_2 from the supraorbital photophore, entering the eye through the aperture between the lens, L, and the iris, I. The fish may see a spot in the visual field cast by light from the supraorbital photophore, if the intensities of R_1 and R_2 are unequal. If the fish adjusts the intensity of R_2 to equal R_1 and controls the output, R_3, of the ventral photophores, VP, accordingly, then the ventral body outline will be camouflaged maximally.

These findings suggest that *Tarletonbeania crenularis* can see a diffuse spot of light cast by the supraorbital photophores in the dorsal visual fields whenever the intensity of the light from the photophore is greater than that of the dorsal visual field. It might be supposed that the fish can perform an incremental threshold discrimination of intensity and adjust the output of the supraorbital photophore until the spot is obliterated. If the fish controls the intensity of light from the ventral photophores accordingly it will not be visible from below against changes in downwelling illumination.

For the photophore system to be maximally effective in camouflaging the ventral body silhouette, a luminescing fish should be horizontally orientated. Observations from bathyscaphes[20,21] show that some non-luminescing mytophids were orientated in positions other than the horizontal.

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Rate of Uptake of DDT from Soil by Earthworms

SINCE 1958, when the occurrence of DDT and its metabolites in the tissues of earthworms was first reported, there have been more than twenty other reports of DDT residues being found in these animals[1,2]. The amounts found have differed greatly, ranging from minute traces to concentrations of nearly 700 p.p.m., although concentrations of the order of 1 to 10 p.p.m. have been more commonly reported. Sometimes the concentrations of DDT residues found in the earthworms have been less than in the soil where they live, although the tissues of the worms have usually contained greater concentrations than the surrounding soil. When all the published data were considered, it was seen that there is a linear correlation between the concentration of DDT (and other organochlorine insecticides) in earthworms and that in the corresponding soil[1]. Not all species concentrate insecticides in the same way or to the same degree[3]; *Lumbricus terrestris* and other species with permanent burrows take up DDT from the soil surface more readily than from residues that have been thoroughly mixed into the upper strata of soil. Conversely, some of the species without permanent burrows, that tunnel haphazardly through the upper 150 mm of soil, take up more residues from insecticides evenly distributed through the upper soil layers to plough depth.

One limitation of the published data is that, in most investigations, residues in samples of soil and earthworms were taken at some arbitrary point in time, without defining the period during which the worms were exposed to soil insecticide residues; so such random samples may represent any stage in what is possibly a long process of absorption and concentration. The rate of uptake or excretion of DDT by earthworms has not been studied.

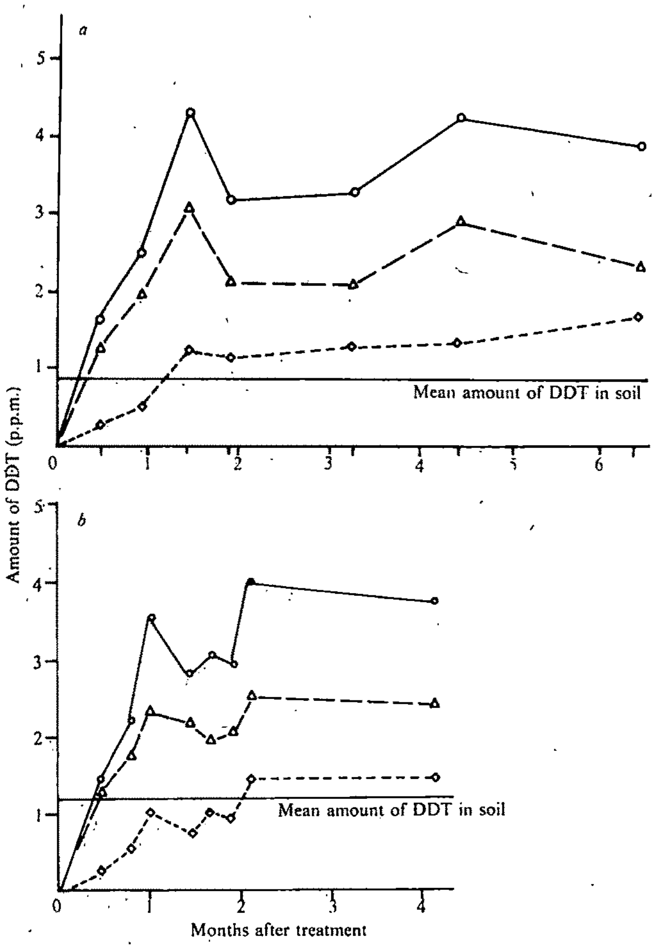

Fig. 1 Results of two series of uptake experiments. ○, DDT and DDE; △, DDT; ◇, DDE.

We therefore investigated the process in a series of laboratory experiments. Uptake of DDT into earthworms was followed in twelve culture boxes (450 mm high × 180 mm wide × 90 mm deep) with wooden sides and perspex backs and fronts that could be easily removed, containing DDT-treated soil. Each box contained ten healthy *Lumbricus terrestris*. This soil, which was free from insecticides before treatment, was passed through a 6 mm aperture sieve to remove stones and debris and was then spread out on a large plastic sheet, sprayed with DDT through an atomiser and thoroughly mixed before being placed in the culture boxes. The dose of DDT was calculated to give approximately 1 p.p.m. ($\mu g\ g^{-1}$) in the soil. Four worms were taken from culture and put separately into Petri dishes containing moist filter paper for 24 h, to allow their gut contents to be voided, then stored at 15° C for several days before being analysed for residues. Small soil samples were taken from each box at the same time, and bulked before extracting the DDT residues.

The excretion of DDT from worms that had lived in soil containing DDT for their entire life cycle was studied by taking sixty such worms from contaminated soil in the field and placing them in culture boxes containing uncontaminated soil. At intervals, batches of four worms were taken at random from the boxes and residues analysed in the same way as in the uptake experiments.

Residues were extracted by grinding frozen worms with four times their weight of anhydrous sodium sulphate, and tumbling for 2 h with a mixture of 100 ml of acetone and 100 ml hexane in polythene-capped jars. The extracts were left to stand until the sediment settled, filtered through a Gooch funnel, transferred to a separating funnel and shaken with 100 ml 2% sodium sulphate solution. The aqueous phase, which settled at the bottom of the funnel, was run off and the remaining hexane/acetone extract stored at 0° C with a little anhydrous sodium sulphate until analysed by gas-liquid chromatography (GLC).

The soil from which the earthworms were taken was analysed for DDT by grinding 50 g of the soil, taken in many small subsamples from the four boxes, with 50 g sodium sulphate, and tumbling in an acetone/hexane mixture for 4 h. The subsequent procedure was the same for the worm extracts.

Small aliquots of the soil or worm extracts were concentrated in a Kuderna-Danish evaporator, and passed through a column of Celite impregnated with a sulphuric acid-oleum mixture to remove most of the interfering substances, but not DDT or DDE which is the principal degradation product. The amounts of DDT and DDE in the final extract were determined by GLC using a stainless steel column (1 m long, 3 mm diameter) packed with 5% SE30 on Chromosorb W and operated at 185° C. An electron-capture detector was used with nitrogen as a carrier gas.

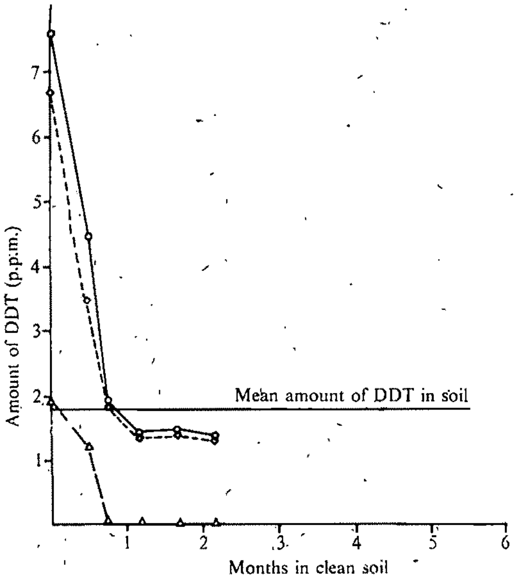

Fig. 2 Results of the excretion experiment. Symbols as in Fig. 1.

The results of the uptake experiments are summarised in Fig. 1*a* and *b* and those of the excretion experiment in Fig. 2. Because the earthworms were progressively removed for analysis or died, it was impossible to continue the experiments for more than 6 months. This period was, however, sufficient to show that worms accumulated the maximum amount of DDT residues from soil after about 1 month; thereafter, although there were small fluctuations, the amounts did not change greatly. There seemed to be three phases—an initial fairly rapid uptake followed by some loss, then a further uptake before equilibrium was reached. Similar double maxima have been reported for the uptake of DDT by other animals such as snails[4] and aquatic organisms[5]. The explanation has generally been that the initial rapid uptake is due to ingestion, whereas the slower phase represents redistribution or residues within the organism, but this needs confirmation. There was evidence that in the box experiment conversion of DDT to DDE within the worms continued and this was confirmed with worms taken from the field; in the latter there was much more DDE than DDT. Moreover, worms placed in untreated soil excreted DDT rapidly, but some of the DDE persisted in their tissues for several months. Some workers have reported larger amounts of DDT than DDE in worms[3,6–8], but others found either the reverse[9] or that there could sometimes be more DDE and sometimes less[10].

There seems little doubt from our experiments that DDE persists longer in worm tissues than DDT. The presence of DDE in worms indicates that they contribute significantly to the initial degradation of DDT, because the amount of DDT converted to DDE in soil is small, possibly only of the order of 10% over several months or even years. During the period of our experiments there was virtually no conversion of DDT to DDE in soil (only traces of DDE being found in the extracts), whereas nearly half the DDT taken up by worms was converted to DDE in 4 to 6 months; and in worms that had been exposed to DDT for a long time, nearly all the residues were DDE.

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Effects of Interrupted Light on Plant Disease

Results of experiments with scab disease on MM109 apple rootstock shoots showed a marked lack of reproducibility, even when done in a glasshouse with some control of light and humidity as well as temperature (the mean visible light intensity was controlled to 1,300±200 cal per cm^2 per month[1], ambient temperatures to 20±2° C during the day and 15±2° C at night with corresponding relative humidities of 70%±5% minimum and 90%±5% maximum). The disease system seemed to be very susceptible to residual fluctuations in the environment. Although this situation could be partly attributed to mechanical failures, examination of results and sunlight radiation records collected over a period of years indicated that erratic and short reductions in sunlight intensity, due to small cumulus clouds passing during the end of the first week after infection, could account for a considerable part of the irregularity. This study, though stimulated by previous observations on apple scab disease, was done with *Venturia inaequalis* and *Podosphaera leucotricha* on MM109 apple rootstock shoots, and strawberry latent ring-spot virus on cucumber plants, in order to investigate the influence of interrupted light on plant disease generally.

Groups of inoculated plants were separated by opaque partitions under a timber-framed hood covered with black polythene to exclude approximately 90% of the daylight, and were illuminated by wide spectrum Kolorarc lamps (Phillips Ltd). Plants received continuous unmodulated light during the day, or were shaded at intervals for 2-min periods by the passage of automatically controlled reciprocating metal shutters, which excluded approximately 90% of the incident light when closed. All plants had at least 12 h of uninterrupted light on the day prior to inoculation. The cucumber plants were subjected to a 16 h day, and when shaded received two, four, or six periods per h every day for 7 d after inoculation because of the rapid onset of the virus disease (see Fig. 1).

Before inoculation of the apple rootstock shoots, the first fully unrolled and partially expanded leaf below each shoot tip was labelled on the petiole and designated Lo, the two leaves above being +1 and +2 and the two below −1 and −2. After inoculation with either *V. inaequalis* or *P. leucotricha*, the following light treatments were applied during 12 h days: (a) unmodulated light for the first 6 d; (b) unmodulated light for the first 4 d followed by 2-min shaded periods twice per h during the fifth and sixth days; (c) as (b) but with four 2-min shaded periods per h; (d) as (b) but with six 2-min shaded periods per h. The plants were subjected to normal glasshouse conditions for the rest of the test period. For both the diseases the numbers of lesions appearing on the five recorded leaves of each shoot ere counted during the tenth to the twenty-first days after fection. Light intensities were adjusted in all experiments compensate for the effects of the shaded periods. Leaf temperature changes in these conditions were comparable with those occurring in naturally modulated sunlight. The mean visible light received by plants while being treated was 30 ± 5 cal per cm^2 per day.

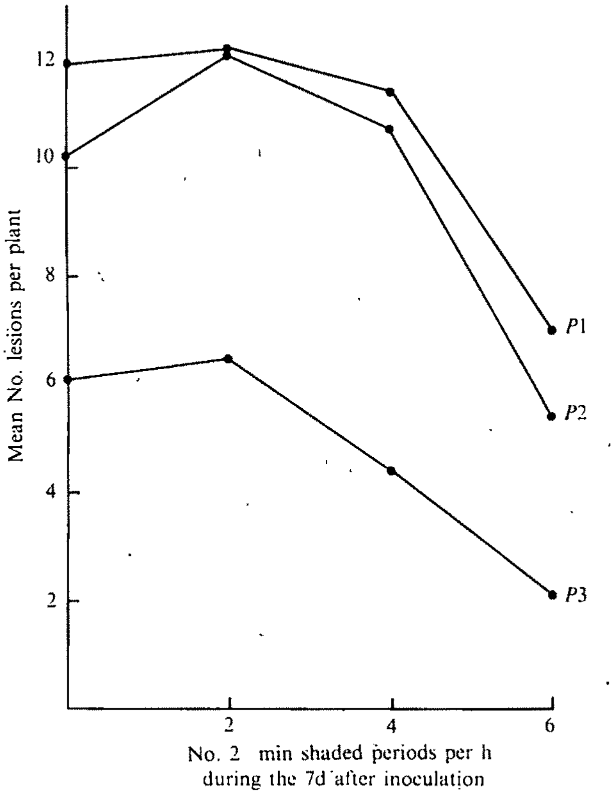

Fig. 1 Inhibition of strawberry latent ringspot virus disease on cucumber plants by the application of six 2-min shaded periods per hour. (Experiment repeated three times for which P_1, P_2 and P_3 are <0.001, respectively.) Cucumber seedlings (cultivar, Ohio), potted singly into 3-inch plastic pots, were inoculated on the cotyledons 7 d after sowing. A strain of strawberry latent ringspot virus (X/XX/XS/SS/Ne Nepo virus group), isolated from blackberry, was maintained in cucumber source plants. Young leaves from plants with symptoms were collected 40 d after inoculation and ground with a pestle and mortar (1 g leaf to 5 ml of 0.03 M phosphate buffer, *p*H 8). The inoculum was rubbed on to cotyledon leaves using the forefinger, plants being taken singly from the different treatment groups in turn, between 1000 h and noon. The cucumber leaves were then washed with distilled water and placed immediately under their respective treatments. Seven days after inoculation a count was made of the number of chlorotic primary lesions, which were best seen by transmitted light. There was no diminution of disease under unmodulated light when the total light received was reduced by the same amount either by day length or light intensity. These effects were readily reproduced.

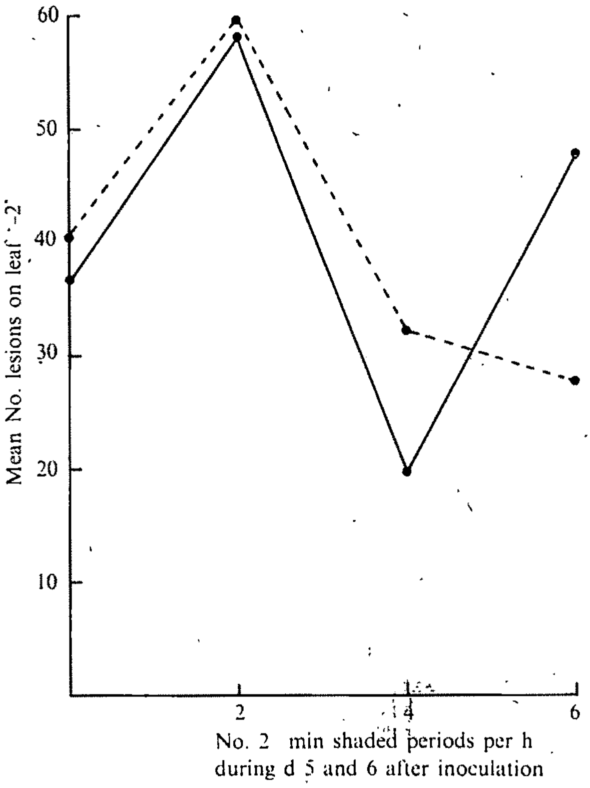

Fig. 2 Effects of interrupted light on the incidence of mildew on apple leaves approaching maturity at the time of infection. ●—●, Control; ● - - - ●, kinetin. Plants were placed in an inoculation tower for mildew infection[2] 24 h before inoculation and removed 18 h afterwards. Inoculum was obtained from four to six heavily infected apple rootstock shoots, to give well-spaced lesions[2]. Kinetin solution (60 mg l^{-1}) was applied to each shoot as a spray at the rate of 3 ml per ten plants, 2 and 3 d after inoculation. The disease was greatest when the shaded periods were applied twice per hour, and least when applied four times per hour ($P<0.001$). Intermediate effects occurred on plants receiving continuous light or six shaded periods per hour during the day. This result was reproducible on either primary or secondary shoots. Kinetin altered the response of the disease to the effects of six shaded periods per hour ($P<0.05$).

The effects of light interruptions on disease reported here, we think for the first time, were impressive yet quite different on each of the systems tested. Experiments with the two obligate parasites gave results which were readily reproducible, in contrast to those given by *Venturia inaequalis.* This facultative parasite, although very susceptible to the way in which light is received, seems to be influenced by other environmental factors which make reproducibility difficult to achieve. The effects of temperature during infection, and of preinoculation conditioning of the plants, are under investigation. The scab and mildew experiments showed the effects of light interruptions to be most marked on leaves approaching maturity at the time of inoculation, though they were visible on all the recorded leaves.

Previous work showed that kinetin can alter both the amount and vertical distribution of the scab disease on the apple shoot, strongly suggesting that the disease is affected by the hormone system of the host[4,5]. The effect of kinetin on apple plants with mildew, when subjected to six light interruptions per hour during the fifth and sixth days after infection, supports the previous suggestion and also indicates that the response to interrupted light is hormone-dependent.

Many plant diseases respond to changes in photoperiod, which in the long term will alter the amounts of available photosynthate. Thus the application of long dark periods both before and after infection affected the growth of bacterial leaf blight on bean, cucumber, cotton and soybean[6].

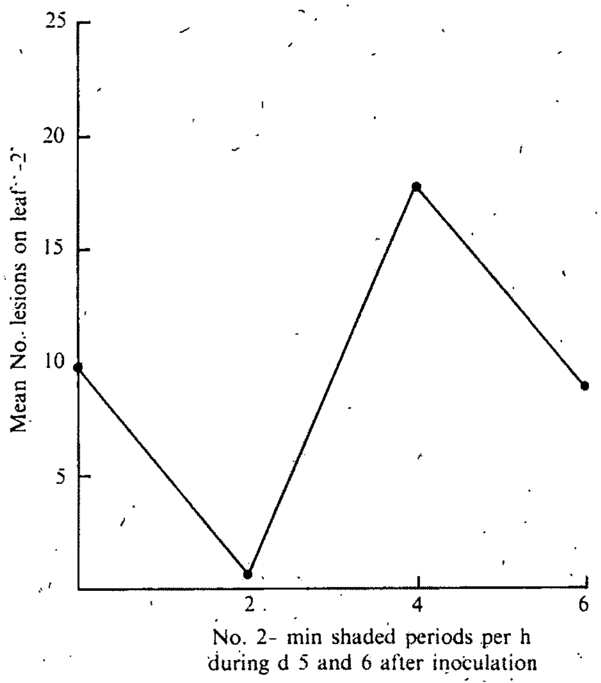

Fig. 3 Effects of interrupted light on the incidence of scab on apple leaves approaching maturity at the time of infection. Cultures of *Venturia inaequalis* (Clone E1)[3] grown on filter paper cylinders standing in 10% (w/v) malt extract solution were used as the source of inoculum in the apple scab experiments. Each shoot was individually sprayed, using compressed air, at the rate of 3 ml per ten plants, the inoculum density being approximately 50,000 conidia per ml[4]. The most striking effects occurred after the application of two, or four, 2 min-shaded periods per hour ($P < 0.001$). Seventy-five per cent of the leaves were apparently immune after receiving two shaded periods per hour and the maximum number of lesions seen on a leaf after treatment was five. By contrast, shading applied four times per hour resulted in only 25% of the leaves showing no disease and a maximum of forty-seven lesions on a leaf.

Young leaf disks inoculated with tobacco mosaic virus produced more virus under the influence of increased light whereas older disks did not respond[7]. The development of *Verticillium* wilt in sunflower was reduced by shortening the day length, but was restored to normal when the light deficit was eliminated by a period of illumination applied during the night[8]. Light also affects the hormone system of plants as indicated by the control of apical dominance in *Phaseolus vulgaris*[9] and by flower initiation in several plant species when light is applied as an intermittent supplement[10]. Since in our experiments the total amounts of light received by the plants were approximately equalised between treatments, thereby reducing variation in amounts of photosynthate, the results indicate that the disease responses to 2-min shaded periods were a function of the hormone systems.

We conclude that the reproducibility of quantitative experiments with many plant diseases will depend upon controlled modulation of incident light, in addition to the accepted standards of light intensity, temperature and humidity. Controlled modulation of incident light may also provide a subtle way of regulating disease development in the study of host–pathogen relations after infection.

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Metabolic Adaptation in the Manic-Depressive

When the manic-depressive is psychologically disturbed, biogenic amine metabolism[1], adrenal cortical activity[2], the disposition of sodium[3] and the concentration of cyclic AMP in the urine[4] may be modified. During normothymic interva none of these measures of endocrine function fall outside normal limits[1–4]. Occasional and temporary diabetic-like responses to a glucose tolerance test appear, however, not only during episodes of depression and mania, but also during asymptomatic periods, despite an adequate carbohydrate intake and with no discernible impact on affect[5]. If, as we have suggested[6], manic–depressives have a defect in the regulation of hormonal balance, an environmentally induced perturbation might make the difficulty apparent even when they are normothymic and their response to a glucose load is normal.

In a healthy man, fasting provokes changes in hormone balance[7] and, probably, changes in the activities of a number of adaptive enzymes[8]. The response to a glucose load is diabetic-like in contrast to the normal response before the fast. A group of manic–depressives was therefore subjected to this simple and reasonably innocuous test of their abilit to reorganise their hormonal balance when faced with a environmental demand for such a change.

Sixteen males volunteered for a 3 d fast. All had glucose tolerances that were non-diabetic as defined by the US Public Health Service[9]. Six were staff members, five were manic–depressives who were normothymic at the time of the experiment, and five were schizo–affectives. The latter groups were being treated with lithium carbonate and were selected from a population whose metabolic and psychological states had been followed for at least 3 yr. Diagnoses were based on the presence of the following symptoms: manic states—hyperactivity, flight of ideas, pressure of speech, elated or irritable mood; depressed states—motor retardation, poverty of thought, depressed mood; schizo–affectives—disintegrated thought and affect and concomitant changes in mood. All of the manic–depressives had a history of at least three manic episodes and two depressed episodes. All of the schizo–

Table 1 Experimental Protocol

Days 1–3	Diet containing at least 250 g carbohydrate. No food after 2200 h on day 3.
Day 4	Pre-fast GTT*—Oral test starting at 0800 h. (Blood samples were collected in the fasting state, then 0.5, 1, 2, and 3 h after drinking a solution containing 75 g of glucose.) No food for remainder of day.
Days 5–6	Fast.
Day 7	Fast GTT*—Repeat oral test starting at 0800 h, then diet containing at least 250 g carbohydrate.
Days 8–9	Diet containing at least 250 g carbohydrate. No food after 2200 h on day 9.
Day 10	Refeed GTT*—Repeat oral test starting at 0800 h.

* GTT—Glucose tolerance test.

Table 2 Age, Weight and Lithium Consumption of Participants

Subject	Age	Li_2CO_3 dosage (mg)	Height (inches)	Weight* (pounds) Pre-fast	Fast	Refeed
Controls						
RH	35	—	73	170	165	167
RT	22	—	71	149	142	145
AB	55	—	68	163	157	160
LV	50	—	75	202	193	197
BM	31	—	76	152	145	148
EF	46	—	67	158	153	156
Manic–depressives						
WG	22	600 bid †	67	143	135	139
AS	30	600 qid †	70	202	194	199
BH	51	300 qid	75	284	272	279
LD ‡	47	—	71	197	187	191
LD ‡	47	600 bid	71	202	191	196
PM	28	600 bid	69	155	149	153
Schizo–affectives						
AM	23	300 qid	66	179	171	175
CW	45	300 qid	69	162	156	160
FD	34	600 bid	74	218	206	213
RB	23	600 bid	72	222	212	219
SE	23	300 qid	71	180	174	178

Participants were weighed just before the glucose tolerance [illegible] on days 4, 7 and 10.

† Bid, twice daily; qid, four times daily.

‡ Patient took test twice: once after he had discontinued lithium carbonate treatment for approximately 3 months; once after he had resumed lithium carbonate treatment for 1 month.

affectives had a history of at least five episodes of mania, hypomania or depression.

The experimental procedure is outlined in Table 1. Participants who had been taking lithium carbonate before the experiment continued their medication in the same amounts and on the same schedule during the fast and refeeding periods (Table 2). This design was selected since several patients were reluctant to discontinue lithium therapy. One patient (LD) was tested with and without lithium. No significant affective changes took place in any of the participants during the experiment.

Serum glucose was determined by the hexokinase: glucose-6-phosphate dehydrogenase reaction[10]. Serum lithium was measured by atomic absorption spectrophotometry and sodium and potassium with a flame photometer. Serum lithium levels increased during the fast but the decrease in serum sodium and potassium was not significantly different from decreases in controls (Table 3). Urine samples were tested for 'ketone bodies' with a commercial preparation that contains sodium nitroprusside and detects acetoacetic acid at a level of 5 mg per 100 ml. A positive reaction appeared on day 6 in all cases and became intense by day 8. No reaction was detected on days 4 or 10.

Table 3 Levels of Monovalent Cations in Serum (mEq l^{-1})*

Subject	Sodium Pre-fast	Fast	Refeed	Potassium Pre-fast	Fast	Refeed	Lithium Pre-fast	Fast	Refeed
Controls									
RH	143	139	143	4.7	4.2	4.4	—	—	—
RT	146	140	144	4.5	4.1	4.3	—	—	—
AB	144	141	145	4.3	4.1	4.2	—	—	—
LV	147	141	146	4.7	4.4	4.6	—	—	—
BM	144	143	147	4.2	4.0	4.3	—	—	—
EF	142	140	143	4.3	4.1	4.3	—	—	—
Manic–depressives									
WG	145	140	145	4.3	4.2	4.4	0.65	0.98	0.58
AS	144	139	143	4.3	3.9	4.0	0.87	1.35	0.78
BH	142	140	143	4.7	4.4	4.6	1.11	1.40	0.83
LD †	145	142	144	4.2	4.0	4.3	—	—	—
LD †	146	142	145	4.3	4.1	4.3	0.73	1.16	0.91
PM	146	140	145	4.5	4.2	4.4	0.98	1.24	0.97
Schizo–affectives									
AM	146	141	145	4.7	4.2	4.4	0.99	1.45	0.91
CW	148	145	149	4.9	4.3	4.8	0.71	1.00	0.61
FD	145	143	146	4.0	3.8	4.1	0.60	1.15	0.64
RB	144	143	147	4.4	4.1	4.3	0.70	1.08	0.68
SE	146	140	144	4.3	4.1	4.3	0.77	1.01	0.73

* Ions measured in a portion of the blood samples taken for the zero time glucose tolerance tests.

† See footnote‡, Table 2.

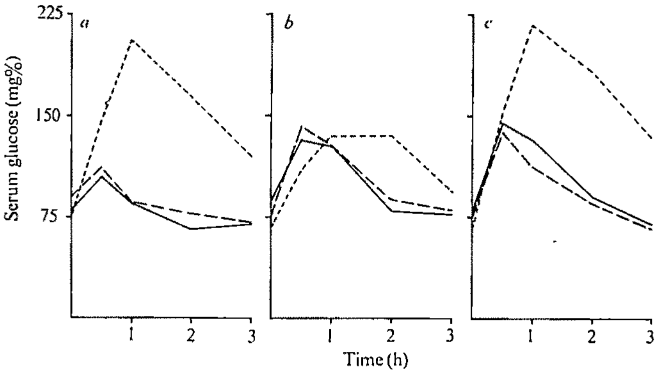

Fig. 1 Effects of fasting on the response to a glucose load. ——, Pre-fast; - - -, fast; — — —, refeed. *a*, Control; *b*, manic–depressive; *c*, schizo–affective.

The results of the glucose tolerance tests are shown in Fig. 1 and Table 4 and, in the case of LD, in Fig. 2. The fast produced notable differences between manic–depressives and the other groups. First, the response to the glucose load, as measured by the sum of the five sampling periods, was lowest in manic–depressives and only slightly higher than their pre-fast levels (36 mg%) while this sum was increased by 306 mg% in the non-medicated group and by 240 mg% in the medicated controls. Second, the half-hour serum glucose levels of the manic–depressives were lower than their comparable pre-fast or refed levels by almost 20%. This contrasts with the relatively higher levels in the controls (35%) and the schizo–affectives (10%) at this time. Third, the manic–depressives showed some adaptation to the fast. Peak glucose levels occurred late (2 h) and levels at this time were higher than in the pre-fast or refed tests. The response, however, was comparatively moderate as well as delayed.

The pre-fast and refed glucose tolerance curves of the schizo–affectives and manic–depressives were somewhat elevated compared to controls. This is consistent with the reports of a higher incidence of diabetic-like responses among manic–depressives[5] and in patients subjected to long term phenothiazine treatment[11], which was the case with the schizo–affectives. Nonetheless, the schizo–affectives reacted

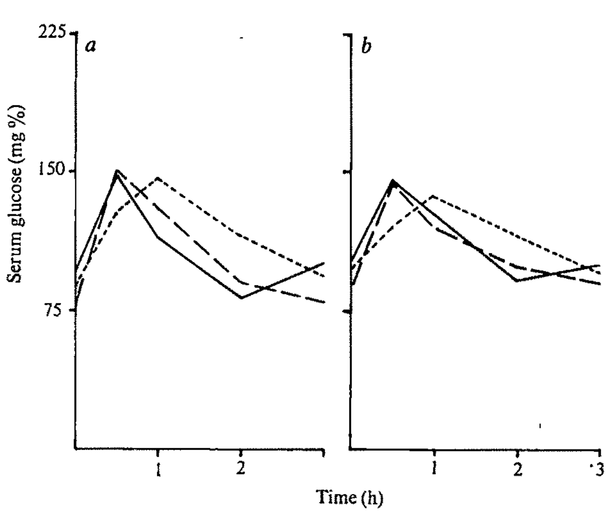

Fig. 2 Effect of lithium on the glucose tolerance curve of a manic–depressive, patient LD (see text and Table 2). ——, Prefast; - - -, fast; — — —, refeed. *a*, Without lithium; *b*, with lithium.

Table 4 Statistical Analysis* of Effects of Fasting on Responses to a Glucose Load

Time (h)	Control Serum glucose (mg%±s.e.m.)	P†	Manic–depressive Serum glucose (mg%±s.e.m.)	P‡	Schizo–affective Serum glucose (mg%±s.e.m.)	P§
			Pre-fast			
0	79± 2.80	N.S.	89± 6.55	N.S.	80± 4.43	N.S.
0.5	109± 7.51	N.S	134± 8.89	N.S.	149± 7.82	<0.01
1	87± 8.59	<0.05	131±14.43	N.S.	131±13.97	<0.05
2	65± 5.86	N.S.	80± 3.98	N.S.	93±11.73	N.S.
3	69± 3.87	N.S.	76± 8.49	N.S.	71± 4.29	N.S.
			Fast			
0	76± 4.69	N.S.	66± 3.29	N.S.	69± 6.34	N.S.
0.5	149±13.27	<0.05	111± 4.71	<0.001	162± 5.51	N.S.
1	206± 7.50	<0.001	137± 7.81	<0.001	216±11.79	N.S.
2	164± 7.88	<0.05	135± 8.28	<0.01	184±12.01	N.S.
3	120± 5.39	<0.05	97± 7.16	<0.01	133± 6.91	N.S.
			Refeed			
0	88± 4.17	N.S.	79± 3.61	N.S.	80± 6.22	N.S.
0.5	113± 9.93	<0.05	140± 4.71	N.S.	138± 6.66	N.S.
1	85± 2.15	<0.01	127±12.91	N.S.	113± 7.16	<0.01
2	76± 5.41	N.S.	91± 4.57	N.S.	86± 7.37	N.S.
3	71± 4.13	N.S.	79± 3.14	N.S.	65± 6.49	N.S.

* All results are expressed as mean and standard error of the mean. A two-tailed Student's *t* test was used for analysis in all cases.
† Manic–depressives compared to controls.
‡ Manic–depressives compared to schizo–affectives.
§ Schizo–affectives compared to controls.

to the fast like the controls.

Though we have not yet found a sufficient number of non-medicated volunteers to establish this with certainty, lithium does not seem to be a critical variable in these experiments. The response of the two lithium groups to the fast was different in spite of the similarity between their pre-fast and refed glucose tolerance curves. The lithium control group responded to the fast like the non-lithium controls. LD's curves were virtually identical with or without lithium.

In further investigations of the glucose metabolism of the manic–depressive, we have noted that the serum insulin levels do not always correlate with the response to a glucose load. While these preliminary data are consistent with a change in overall hormonal balance rather than a change in any single hormone, the complexity of the neuroendocrine system presents problems in the identification of the critical difficulty. Hormonally-mediated responses involve synergistic and antagonistic effects on rate-limiting cellular processes[12], feedback regulation of hormone levels[13], influences on gene expression[14] and protein synthesis[15] and a critical role of the central nervous system in the organisation of these events[16]. Glucose metabolism itself is influenced by insulin, glucagon, catecholamines, glucocorticoids, thyroid and growth hormone[17].

Further, Britten and Davidson[18] have suggested that the expression of mammalian regulatory genes[19] is under hormonal control. A defective regulatory gene could account for the unusual response of the manic–depressive to a fast and still permit successful and appropriate responses to the many stimuli which continuously provoke changes in hormone balance. Since long-lasting modifications in gene expression can be environmentally induced[20,21], such a defect in function does not necessarily imply a mutation.

Whatever the origin of the unusual response to the fast, it appears that the manic–depressive is at risk biologically, a view which finds support in the report of the premonitory changes in noradrenaline metabolism in manic attacks[22] and the failure to discover immediate precipitating psychological causes for the sudden appearance of a disordered affect[23]. It is also apparent that susceptibility to severe affective episodes occurs in individuals with very different biochemical characteristics, a fact which may be of value in diagnosis. Whether the hormonal disturbances associated with a disordered affect in manic–depressives and schizo–affectives are identical or whether differences occur here also is now subject of investigation in this laboratory.

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book reviews

Newton's *Principia Mathematica*

Philosophiae Naturalis Principia Mathematica. By Isaac Newton. Third edition with variant readings. Edited by Alexandre Koyré and I. Bernard Cohen, with Ann Whitman. Vol. 1 pp. 547; vol. 2 pp. 368 (Cambridge University: London, August 1972.) £25.

ntroduction to Newton's Principia. By I. Bernard Cohen. Pp. 380. (Cambridge University: London, October 1971.) £13.

NEWTON'S *hypotheses non fingo* is almost as well known as his experiments with the prism or his observation of the falling apple. But its meaning is not so clear. Not that the eighteenth and nineteenth centuries were in much doubt about it. In English it meant "I frame no hypotheses" in the words of Andrew Motte's English translation of the third edition of the *Principia*, and in French it meant the slightly different "J'imagine point d'hypotheses" in the words of Voltaire's friend Emilie du Châtelet's posthumous French translation. For almost everyone—especially scientists—it meant facts are safe, hypotheses dangerous, hold to the former, avoid the latter and, in so far as you do that, you will be following the immortal Newton's advice and the success—if not the importance—of your enterprise will be guaranteed.

Newton's practice, however, did not always square too well with this advice. Admittedly, there are no very obvious hypotheses in his first optical paper of 1672, that supreme example of the art of drawing deductions from experiments. But what of the paper of 1675, "An hypothesis explaining the properties of light . . .", with its fantastical explanation of reflection and refraction in terms of an hypothetical ether whose pressure was greater in air than in solid matter, and to which was later joined an *ad hoc* hypothetical "skin" at the inter-surface between air and solid, not to speak of "easy fits" of transmission and reflection to explain partial reflection and the colours of soap bubbles and of Newton's "rings". Newton was evidently prepared to "entertain" hypotheses, even if he did not "frame" them, and even if he only entertained them for the sake of certain great "virtuosos" whose heads ran "much upon hypotheses".

But what of the *Principia* itself, that arc of the scientific covenant? Even if we suspend judgment on the charge of an hypothetical element in concepts such as force, mass or gravitational attraction with their inextricable mixture of the actual and the ideal, it is impossible to deny the existence of no less than eight named hypotheses at the beginning of Book 3 of the first edition. But in this edition the *hypotheses non fingo* is absent; it appears first in the famous concluding *Scholium Generale* of the second edition. In that edition the number of named hypotheses is reduced from eight to one. In other words, a comparison between the first and second editions of the *Principia* indicates a considerable hardening in Newton's attitude to hypotheses. So that whatever he actually meant by *hypotheses non fingo* in 1713 it is not likely to have been exactly the same as what he would have meant by the same phrase in 1687.

This discovery, made independently by I. B. Cohen and the late Alexandre Koyré, had two important effects: it led to a fundamental reappraisal of Newton's attitude to hypotheses, and to the suggestion by Koyré—since generally accepted—that what Newton really meant by *hypotheses non fingo* was not "I do not frame hypotheses", but "I do not feign hypotheses"—in the manner of Descartes, for example. In the second place, this discovery led to the realisation that Newton's thought did not suddenly become static after the publication of the *Principia* in 1687, and that the various editions of the *Principia* contained a great deal of evidence—hitherto largely ignored—on his changing attitudes to certain key concepts including that of hypotheses. Like some of the best discoveries this one is inclined to seem obvious in retrospect. But it fired Koyré and Cohen to undertake an enterprise of heroic proportions, a variorum edition of the *Principia.*

Of the variorum edition itself I do not propose to say much beyond praising its admirable plan, its beautiful printing and layout, and its excellent critical apparatus. The edition is based on a total of eight texts, those of the three editions, the printers' copy of the first edition, and the four interleaved copies —two each for the first and second editions—kept by Newton himself for the noting of errors and improvements. The level of accuracy attained in such a work is evidently of cardinal importance. This is something which can only be determined by use over a long period. I have no doubt it will be found to measure up to the most exacting standards.

The accompanying introduction by I. Bernard Cohen is mostly taken up with the history of the preparation of, and reaction to, the three editions of the *Principia.* Of this history a great part is devoted to the story of the long and gradual process of correction and emendation by which the first edition of 1687 was transformed into the greatly improved second edition of 1713 from which the third edition of 1726 differed little. Apart from Newton himself, pursuing the endless task of correction with unswerving and unhurried determination, there appear a number of other figures. Halley, who had played such a large part both in the composition and publication of the first edition, and who wrote the first review of it. The Italian Fatio De Duiller to whom Newton seems to have been powerfully attracted for a time before his scientific judgment reasserted itself, and whose notes on the *Principia* are interesting not for their intrinsic merit, but for what they tell us of the early stages in the preparation of the second edition. David Gregory, who made a more intensive study of the *Principia* than anyone apart from Halley and Newton himself, but who was disappointed in his plan to publish a commentary, or possibly even in becoming editor of the second edition. Richard Bentley, under whose masterful hand the printing of the second edition was actually begun in 1708, only to be discontinued after eight pages, probably because Newton suddenly realised that Bentley's mathematical inadequacies would have necessitated his checking all the proofs himself.

Finally, the somewhat tragic figure of Roger Cotes, Plumian professor of mathematics at Cambridge, who had the necessary mathematical ability, the tact not to offend Newton, and the strength of character to stand firm against him when he knew he was in the right and Newton in the wrong. As the Newton-Cotes correspondence shows, Newton's debt to Cotes was enormous as regards his overseeing of the printing, his discovery of errors, suggestions for improvements, and provision of the famous preface with its brilliant exposition of the Newtonian philosophy of science including Newton's very subtle attitude to gravitational attraction. Sad to say, as Professor Cohen admits, there is no trace in the Newton-Cotes correspondence of any word of thanks from Newton to Cotes for all his enormous labours: and Newton's famous epitaph to Cotes following his early death in 1716: "If Mr Cotes had lived we might have known something" seems to imply that Newton never fully appreciated Cotes' contribution, since but for Cotes the second edition of the *Principia*, if it had ever appeared, would certainly have been a much less important edition than it actually was.

Throughout almost the whole of the introduction Cohen shows an easy mastery of a vast mass of source material, follows the good example of Newton himself in the matter of English prose style, and strikes a happy balance between an uncritical reproduction of every scrap of relevant information and a too shallow and superficial treatment. Only in chapter 3—"Steps toward the *Principia*"—does he seem to falter a little. One reason for this may have been uncertainty what to say here about the technical, dynamical aspects of the early steps in the composition of the *Principia*, about which an additional volume of commentary is promised. In fact the treatment given is not only confused on occasion, especially in the discussion of the various versions of the tract *de Motu*, but it also contains a number of somewhat hasty judgments which will hardly command universal assent.

Thus it seems to me somewhat improbable that in 1684 Newton was admired by the *cognoscenti* not only for his discoveries in mathematics but also for his "grasp of the fundamentals of dynamics" (page 47); in the first place Newton was very secretive about his early work in the subject, and in the second place "the fundamentals of dynamics" had, in part, still to be formulated. Equally suspect is the assertion (page 52) that Newton "apparently learnt of Kepler's law of areas in 1678 *and at once was able to solve the problem of Keplerian planetary motion*" (my italics). This view, originally put forward by D. T. Whiteside[1], was very improbable *a priori*, and was then effectively ruled out by J. L. Russell's[2] demonstration that Kepler's laws had been much more familiar to astronomers in the first half of the seventeenth century than had previously been thought to be the case—Russell lists four authors, Herigone, Ricciolli, Boullian and Wallis, who all cited Kepler's second law in its exact, areal form before 1660. Admittedly, the second law was evidently the least familiar, and its dynamical significance remained entirely hidden before 1679. But it is stretching credulity to suppose that Newton had not noticed—and deeply pondered on—this extraordinary law before 1679. Curious, too, is the assertion (page 53) that "only near or at the surface of the Earth did he (Galileo) conceive of a true linear inertial motion" or the truly extraordinary remarks (page 61) that the tract *de Motu* "does not yet bear the mark of genius" and contains "no hint of the grandeur of the *Principia* to come": after all, it was in this tract that Newton first solved the problem of Kepler motion, the very cornerstone of the whole *Principia*.

Critics will inevitably differ, as critics do, on these and other minutiae. All will be agreed, however, on the magnitude of the achievement of the variorum edition and its introductory volume. Barring the unlikely appearance of important new Newton manuscripts, the whole work will surely remain definitive well into the next century, if not beyond. Alexandre Koyré, I. B. Cohen and their band of devoted assistants have deserved well of the republic of letters, and I can only regret that Koyré did not live to see the completion of the work originally conceived by him and Cohen in 1956. J. W. Herivel

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Rockets and roubles

The Origins and International Economics of Space Exploration. By Bernard Lovell. Pp. viii+104. (Edinburgh University: Edinburgh, 1973.) £1.50.

It is excellent to find such a wealth of information in so slim a volume. I especially enjoyed the succinct and clear historical part (the first quarter of the book). To describe the early military history of the rocket, to disentangle the labours of Tsiolkowsky, Goddard and Oberth, to demonstrate the independence of Soviet work from the earlier German efforts, to explain the administrative tangles in the United States before NASA got going, and all in some twenty very readable pages is most valuable. Much of the rest of the book gives data on costs and on numbers of launchings, and examines the relative sizes of civil and military space efforts in both United States and Soviet Union all in very convenient and easily assimilable form, and describes briefly, and perhaps a bit conventionally, the various benefits of space activity. Science in particular is dealt with too briefly for anybody to learn much from it, but more justice is done to the applications. The contrast of the cost of manned and unmanned space activities is well brought out, and a somewhat implicit admiration, richly deserved, for the political vision, superb decision taking, and technological brilliance of the Apollo programme is understandably followed by a much more dubious attitude to later manned activities (Skylab and Shuttle) to which so much highly promising space science (high energy astronomy and planetary observation) is being sacrificed.

However much I admire these concise descriptions, I am distinctly less thrilled by Sir Bernard Lovell's more philosophical comments, comments that are far too brief and therefore lack clarity and depth. If there was not the room to develop the argument more fully (which might well have been fascinating), then the commentary should have been left out. As it is, the reader is simply confused and irritated by the sputtering that first points one way, then the other, never addressing itself to any problem for long enough, never resolving the contradictions.

To give some examples: after soberly describing the V1 and V2 campaigns, and the casualties caused, he rightly says that these weapons could have affected the war far more but for the bombing of Peenemunde, only to be "filled with dismay and an unresolved conflict" by the thought that this bombing was made possible by the blind bombing aid which he had helped to develop "illuminating for destruction the genesis of one of the greatest scientific developments of all time . . .". His dismay is hard to understand, given the facts of the situation. Similarly, on page 86 he stresses that the United Kingdom's space expenditure was so minute that, if transferred to education, health and social security, it would have hardly made any difference, but on page 87 he speaks of wasting "these extremely large sums" (that is, those applied to space, perhaps including this time the other European countries). Whether depending on the United States for launching rockets (page 87) is the bad thing it is implied to be at least deserves to be debated. I, too, am keen on space, but would find it hard to sustain (though I would enjoy reading a good argument on the issue) the assertion that "many of the urgent problems associated with the predicted doubling of the world's population can be alleviated by space applications" (page 88). These are important and controversial issues on which reasoned argument would have been most welcome, but little benefit is derived from over-brief and often unclear assertions.

But my gripe about what is not in the book should not cloud my advice to all interested in space to read it for its factual content. H. Bondi

Food and development

The Nutrition Factor: A Study jointly by the Foundation for Child Development and the Brookings Institution. By Alan Berg. Portions with Robert J. Muscat. Pp. xii+299. (Brookings Institution: Washington DC; Allen and Unwin: London, August 1973.) £4.50 boards; £1.75 paper.

THIS is a worthwhile book. It is concerned with the improvement of nutritional health in the developing countries but differs from most other books on the subject in that it goes to great length to point out that such improvements have an important role in national development. This is not a practical manual in that it does not set out step-by-step instructions—the approach is not so dogmatic; rather it deals in a philosophical way with the background to the problem and suggests principles for effecting change. The book will surely become essential reading for students of applied nutrition and for those administrators and civil servants in the developing countries who have need to convince their rulers that the malnourished members of the community are not economically insignificant.

The starting point of Dr Berg's philosophy is best summed up in his own words: "malnutrition adversely affects mental development, physical development, productivity, the span of working years—all of which significantly influence the economic potential of man". It would be easy to quibble with the opening chapters in which Dr Berg justifies this statement. Perhaps too little theoretical proof is available but few scientists or doctors would doubt its basic truth, and the aim of the book is to encourage national action rather than stimulate further fundamental research.

A whole chapter is devoted to the cynical but often encountered argument that improved nutrition will merely exacerbate the population problem. The nutritional adviser will need to combat this line of attack; the chapter provides some valuable ammunition. People in developing countries will not readily accept contraception until they can be sure that their children, in particular sons, will survive.

Dr Berg quite rightly believes that significant nutritional improvements at a community level will materialise only if the problem is viewed from a national perspective and tackled by broadly based preventive measures. He points out the limitations of the "person to person diagnostic and curative approach"; however essential this may be to the ministries of health, national planners need to adopt a more comprehensive policy. The main possibilities discussed are: economic growth leading to higher incomes and hence better nutrition, improved agricultural production and farming technology, nutritional education, the formulation of new foods and nutrient fortification, the encouragement of new food industries and, finally, feeding children through nationally organised public programmes. Each topic is dealt with and commented on in an instructive manner. The conclusion is that each approach has its valuable features but none can achieve the desired goal alone; an integrated plan is essential.

The main stimulus for Dr Berg's approach to malnutrition has been his experience of India, and the narrative ends with a discussion of the lessons learnt there both from the general situation and from the crisis brought about by the Behar famine. Dr Berg considers that the beginnings of a national programme in India can be looked upon as a prototype by other nations.

The book concludes with numerous tables and appendices providing valuable facts and figures together with a comprehensive bibliography.

This monograph purposefully confines itself to the food and dietary factors involved in malnutrition and readers might, if they did not read carefully the penultimate paragraph of the first chapter, be led to believe that these were the only ones that matter. Perhaps it is of value to dispel this idea by again quoting Dr Berg: "In a systematically planned search for remedies to nutritional problems, many non-standard nutritional measures will be considered. Protected water supplies, waste disposal and other forms of environmental sanitation, immunisation against certain diseases, for instance, all relate to the incidence of malnutrition, its relative severity and the reduction of mortality. Although such measures are not normally thought of as nutritional measures, they may be significant determinants of nutritional status, or, more likely, essential in combination with the food-related measures that this study focuses on".

These words emphasise still further the enormity of the problem facing those who are trying to improve nutritional health in the developing world. Little wonder Dr Berg advocates such far-reaching preventive measures; his book is timely and warmly welcomed.

R. G. WHITEHEAD

Solid state electrochemistry

Electrochemistry of Metals and Semiconductors: The Application of Solid State Science to Electrochemical Phenomena. By Ashok K. Vijh. Pp. xiv+297. (Dekker: New York, May 1973.) $23.50.

THE electrode-solution interface has been studied from the viewpoint of solid state physics by many workers since the pioneer paper of Grimley and Mott in the 1947 Faraday Discussion. During these years the theory of redox reactions at metal or semiconductor electrodes has been developed by Gerischer on the basis of the classical hydrogen overvoltage paper of R. W. Gurney; a good many practical applications have been examined, and some established in industry. Dr Vijh works in the Hydro-Québec Institute of Research, and has published a number of papers in recent years. His book sets out to provide first a rudimentary introduction to the solid state (really band theory) to electrochemists unfamiliar with this topic. There follow eight chapters, including three on electrode reactions (general principles, elemental semiconductors, bulk compound semiconductors), then anodic oxidation of metals and non metals, and the effect of surface films on electrode reactions. In chapter 7 the solid state properties of metals are related to their electrochemical behaviour, and in chapter 8 electrochemistry in the solid state (as solid electrolytes and proton conduction in ice, and so on) is studied. A final chapter deals with industrial aspects, some ten applications being noted from fuel cells and batteries to mineral processing.

Most of my criticisms turn on points of detail, since the book as a whole usefully fills an important gap in the monographic literature. To say that semiconductors show a non-Ohmic behaviour under ordinary conditions (p. 21) is surely rather misleading. In view of its importance more attention should have been given to explaining the Fermi level, and its relation to energy bands and impurity levels. The printing of the various Galvani potentials on p. 191 *et seq.* leaves much to be desired. On p. 21 intrinsic conductivity is rightly written as $\sigma_0\ \exp\text{-}(E_g/2kT)$, on p. 131 wrongly as $\sigma_0\ \exp\text{-}(E_g/kT)$. There is published evidence for the free energy of activation for oxide growth proportional to the oxide thickness (p. 132) but this is not quoted.

Putting such detailed matters on one side, the book is easy to read, and should perform a valuable function in introducing research workers to an important new field of interfacial activity, and we may expect it to stimulate further efforts in this direction. Biological systems are rightly excluded from the present volume, but there are also signs of a growing activity in this area.

D. D. ELEY

Animal chromosomes

Animal Cytology and Evolution. By M. J. D. White. Third edition. Pp. viii+961. (Cambridge University: London, June 1973.) £19; $55.

WRITERS of general accounts of chromosomes before Professor White used to attempt to cover both plants and animals. This policy was justified by the uniformity in principle and also by the complementarity in detail of the two kingdoms: the chromosomes of plants were more useful in showing the causes of evolution; those of animals revealed above all its consequences. Or, to put it in another way, the immense range of experiments, chemical and physical, genetic and physiological, that have been successful with plant chromosomes provide quite a different picture from the descriptive variety which is revealed by a systematic survey of animal chromosomes.

Professor White was the first, in successive editions of this book, to try to disentangle the animals from the plants, the consequences from the causes. He took this course, no doubt, because the animals suited him extremely well. He is the most experienced specialist in animal cytology in the world and he excels in describing and illustrating the wealth of variation of chromosomes in their form and behaviour as they operate the multifarious genetic and developmental systems of animals.

Naturally Professor White has to refer to the principles of chromosome behaviour revealed by plants, but he does so reluctantly and piecemeal, lightly suspending his judgment whenever the issue becomes serious. Evidently he does not recognise the contrast or the complementarity between the opposed evolutionary aspects of chromosome study, and no one from reading this book could pick up any such disturbing notions. There are many students of chromosomes, to be sure, who will not want to be disturbed, and for them Professor White has provided a solid and satisfactory work of reference.

C. D. DARLINGTON

Scottish shores

The Coastline of Scotland. By J. A. Steers. Pp. xx+335 (64 plates). (Cambridge University: London, 1973.) £10.50; $31.50.

IT is now almost thirty years since Professor Steers's monumental work on the coastline of England and Wales appeared. The natural sequel was a similar study of the coasts of Scotland and now, in retirement, he has, with characteristic thoroughness, set out his views on the form and origin of some of the finest coastal scenery in Britain. The book is cast in much the same mould as his earlier work with a few introductory chapters covering general themes to be followed by a survey of the whole coastline, including all the offshore islands. Inevitably he has drawn heavily on the work of others, for although he has walked over nearly all the coastline he describes, detailed studies of individual sections proved impossible.

Fortunately the geological memoirs of Scotland are particularly detailed as regards coastal sections and many of the early volumes, like that of Caithness, contain a wealth of physiographic information. There is, however, a general lack of research papers similar to those found in the local journals in England. In part this deficiency has been made good by the research efforts of Scottish geographers, particularly those based at Aberdeen. Professor Steers has used their studies to advantage in his text to provide local colour for what is basically a factual account.

Undoubtedly the book will prove of great value to the specialist but seems hardly likely to make easy reading for the amateur enthusiast with an interest in our coastal setting. It is to be regretted that Professor Steers has not felt able to draw upon his vast experience to be more speculative, not only posing problems but giving a lead as to their solution. For the island of Ailsa Craig, for example, he contents himself with a factual account of its geology and vegetation (pages 103–4) without real reference to the interesting ness feature which dominates its eastern coastline. Again in the entrance to Inverness Firth he makes detailed reference to the classic work of Ogilvie, carried out over fifty years ago, when the relative roles of tidal currents and wave action were appreciated for the first time. Since then much new evidence has been forthcoming on this important aspect of coastal evolution, particularly in relation to sediment transport by residual water movements. This is a theme which Professor Steers could have properly developed without recourse to detailed research, for the evidence is there in the bottom configuration of the firth.

The book is well illustrated with maps. It has a centre spread of excellent photographs from the Cambridge University collection of air views, although as they do not occupy the full page size they give the book an old fashioned appearance. Inevitably a comparison will be made with his earlier book on the coastline of England and Wales. It is a sad fact that as book prices have risen steeply in the last few years the quality of production has declined.

A. H. W. ROBINSON

Sea urchin development

Development Biology of the Sea Urchin Embryo. By Giovanni Giudice. Pp. x+469. (Academic (Harcourt Brace Jovanovich): New York and London, August 1973.) $32.

THERE is a great need for more sympathetic communication between experimental embryologists and molecular biologists; and this book, by drawing together the enormous amount of information on the morphogenesis, ultrastructure, biochemistry and molecular biology of the sea urchin embryo, makes a timely and scholarly contribution towards this end.

For the reader with no formal background in embryology there is a lucid account of the experiments of the early 1900s that still provide the basis of current ideas about the programming of sea urchin development; for example, the proposed existence of gradients of potentiality to form ectodermal or endodermal structures emanating from the 'animal' and 'vegetal' poles of the egg respectively. There is fascinating reading, too, in the descriptions of sperm penetration, the behaviour of the male and female pronuclei, and the membrane changes following fertilisation—examples of phenomena which, as yet, have no molecular explanation.

The reasonably up to date summary of research on the sea urchin embryo from a more biochemical viewpoint contains a clear description of experiments which led to such ideas as th existence of 'masked' maternal messenger RNA, and the synthesis of large amounts of histone and histone messenger RNA by the early blastula. But this is a rapidly moving field and it is a pity that the book was finished (early 1972) before the application to the sea urchin embryo of important new advances in the technology of isolating and translating messenger RNAs (see, for example, *Nature*, **245**, 8; 1973) could be included.

Dr Giudice's book is also a useful source of practical information about handling embryos, separating cells, problems of permeability, and so on. It is clearly written, with paragraphs summarising the main problems to be solved and the information available. Beyond this synthesis, however, the author makes no attempts to provide new insights or theories. Finally, the reader is left in no doubt as to the disadvantages, as well as the advantages, of the sea urchin in studying problems of developmental biology, for Dr Giudice is no slavish devotee of his research material. The main disadvantage is, of course, that no genetic analysis of development can be made because it is very difficult to rear the embryos in the laboratory beyond the pluteus stage.

BRIGID HOGAN

matters arising

Douc Langurs

SIR,—Relevant to Dr Kavanagh's observations (*Nature*, **239**, 406; 1972) of food sharing among captive douc langurs (*Pygathrix nemaeus*), I should like to describe some of my own observations of these monkeys in the wild. Between May 1967 and May 1968, I made fourteen visits to Mount Son Tra (elevation 96 m above sea level), about 10 km north of Da Nang on the northeast coast of South Viet Nam. Here I watched two groups of douc langurs as they fed in the canopies of the trees which they inhabited. At the time the animals were relatively tame, benefiting from partial protection afforded by being on a military base, but recently they have declined on Son Tra (Van Peenan *et al.*, *Mammalia*, **35**, 127; 1971). The first groups consisted of four adults, apparently one male and three females (distinguishable when external genitalia were visible), and a baby. The second group consisted of five adults (apparently two males and three females), a half-grown monkey and a baby. The members of a group foraged in close proximity, usually within 10 m of each other, although not always in the same tree, and they rarely came to the ground. Often two or three animals fed in the same terminal clump of foliage, and occasionally when one pulled down a branch, an adjacent individual also fed on it. This type of passive food sharing was noted by Kavanagh.

I also observed active food sharing on two occasions. In the first group of langurs an adult female bit a small section of leafy twig and passed it to the male who quickly stripped off and ate the leaves. The two animals continued to forage within 2 m of each other, but no further interaction was noted for about 30 min. Then, however, the female solicited copulation from the male who subsequently mounted her, but the animals were disturbed by a passing vehicle before intromission was achieved, and they disappeared from view. In the second group I saw a similar case of food being passed from an individual of unknown sex to a female who had a baby clinging to her. I observed no further interaction between these two animals in the few minutes before they disappeared in the foliage.

In each case the passing of food seemed to be spontaneous. The recipient did not appear to threaten or beg, although as Kavanagh pointed out there may be subtle communication patterns inapparent to the human observer. Further observations of these animals in the wild may reveal, as Kavanagh suspected, that food sharing is not rare in family groups in natural conditions. I should add that the species is endemic to the Indochina Region, including Hainan Island, where much of its environment has suffered profound changes, and it is considered an endangered species[1].

Yours faithfully,

MICHAEL GOCHFELD

Division of Environmental Health Sciences,
Columbia University School of Public Health,
New York, New York 10032

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[1] *Red Data Book: Mammalia* (IUCN, Morges, 1972).

obituary

Frederick James Dent

FREDERICK JAMES DENT, FRS, OBE, leader of the 1971 MacRoberts Award winning team, died on October 5 at his home in Malta after a short illness. He was 67.

Dr Dent was born in 1905 and was educated at the Modern School, Leeds. He was a student at the University of Leeds under Professor J. W. Cobb, CBE, obtaining his doctorate in 1929 for an original piece of work on the carbon steam reaction. In the same year he was appointed research chemist in charge of gas production investigations carried out by the Joint Research Committee of the Institution of Gas Engineers and the University of Leeds. In this period he conducted a classical study of the water gas process on the full scale which led to a detailed laboratory investigation of the gasification of coal in steam and oxygen at high pressure.

It was during this latter work in 1937 that he discovered that methane produced during gasification under pressure was formed by the direct action of hydrogen on the coal substance. During this same period he carried out a study of the catalytic synthesis of methane from carbon monoxide and hydrogen and identified and solved the problems associated with the application of this reaction to the production of town gas. Both these discoveries, the one relating to coal hydrogenation and the other to methanation, have currently enormous significance in the work going on today in the United States to counter the growing natural gas shortage by the gasification of coal to methane as a substitute for natural gas.

On the day of his funeral the successful start-up of a methanation demonstration plant at the coal gasification plant of Scottish Gas, at Westfield, was announced in the press. The previous week this plant, the essential features of which are directly based on Dent's work, produced the first substitute natural gas from coal on a commercial scale, forerunning new plants in the United States which will produce billions of cubic feet of synthetic natural gas per day in the future. The new generation of processes now being developed in the United States for the production of substitute natural gas will practically all also incorporate the coal hydrogenation reaction which he discovered at Leeds and first demonstrated on a pilot plant scale at the Poole Research Station of the Gas Research Board .

It was at Poole, where he was Assistant Director of the Gas Research Board from 1943–51, that many of Dent's studies on gas production processes, both paper and experimental, were initiated. For example, it was here that the first coal and oil were hydrogenated in fluidised beds and methanol was first catalytically gasified. It was not until he was appointed Director of the Gas Council's Midlands Research Station in 1951, however, that the funds needed to enable him to develop his ideas to commercial reality became available.

The move to Birmingham and later to Solihull coincided with the start of a change in the fuel situation and a shift in emphasis from coal to oil as a feedstock for gas production. The work on the hydrogenation of oil to gaseous hydrocarbon—rich gas accelerated with the construction of a large fluidised bed hydrogenation pilot plant, followed later by two more specially designed ones which led to the development of the Gas Recycle Hydrogenerator. This process, designed specifically to hydrogenate distillate oils, was quickly adopted by the industry and more than fifty units were installed in this country and abroad for the enrichment of over one billion (10^9) cubic feet per day of town gas.

At the same time, he pursued the development of the Catalytic Rich Gas (CRG) process and, with a highly effective catalyst, brought this also successfully to the commercial stage. Some forty-four units of CRG-based town gas plants have been built, ten of them abroad, while more recently two streams of CRG-based substitute natural gas plants have been commissioned in the United States with a further nine streams in various stages of construction. A further plant is shortly to start up in Japan. The combined thermal output of all these plants, some of which are the biggest in the world, matches the size of the present British gas industry.

During this period of intense activity on oil processes, Dent found time to extend the development of the Lurgi fixed bed high pressure coal gasifier to slagging operation. Two pilot plants were built and operated in the late 1950s and early 1960s and the world's first high pressure steam and oxygen slagging gasifier was satisfactorily developed and demonstrated at Solihull to prove the potential advantages which Dent had predicted for this system from his earlier laboratory studies at Leeds. Increasing interest is currently being shown in this gasifier by companies and government agencies concerned with coal gasification in the United States.

Dent's outstanding contributions to the development of processes for the gasification of coal and oil received worldwide acclaim. His work was recognised by the award of the Birmingham Medal (the highest award of the Institution of Gas Engineers) the Melchett Medal of the Institute of Fuel, the Walton Clark Medal of the Franklin Institute of Philadelphia and the Coal Science Medal of the British Coal Utilisation Research Association. He was awarded an OBE in 1958 and was elected a Fellow of the Royal Society in 1967. These honours culminated in 1971 with the engineering profession's highest prize, the MacRobert Award, which he received jointly with the chief members of his team.

His success stemmed perhaps mostly from his ability to interpret his creative scientific work into practical chemical engineering and process technology. His unbounded enthusiasm and the real enjoyment he had in his work, however, produced the drive in others which was essential to the rate of progress he achieved, a factor which he always generously recognised. He was modest to a fault, impatient with the less well informed and demanded complete technical integrity. Amongst those with a common interest in gasification and gas production processes he made many friends who will retain a memory of a cheerful and kindly man with a deep and detailed understanding of both the theoretical and practical aspects of his subject.

He leaves a widow, a son, a daughter and three grandsons.

Announcements

Appointments

Professor A. D. Bradshaw is to be chairman of the **Nature Conservancy Council's** advisory committee on science.

Derek A. Ratcliffe has been appointed Chief Scientist of the **Nature Conservancy** and **Ian Prestt** is to be Deputy Director.

Professor W. A. Holmes-Walker has been appointed Director of the **British Plastics Federation.**

Harry Hookway, Chief Executive and Deputy Chairman of the **British Library** has been elected President of the Institute of Information Scientists.

Joan Eileen Walsh has been appointed to be Professor of Numerical Analysis of the **University of Manchester.**

Miscellaneous

The Lomonosov Gold Medals for 1973 have been awarded to **Academician Aleksandr Pavlovich Vinogradov** for his work in geochemistry and to **Vladimir Zoubek** for his work in geology.

The Council of Systematics Association Department of Biology plan to offer an Essay Prize in 1974.

Erratum

In the article "Pharmacologically induced behavioural supersensitivity to apomorphine" by Daniel Tarsey and Ross J. Baldessarini (*Nature new Biol.*, **245**, 262; 1973) the following sentence should be added. "Dr Tarsy is a member of the Department of Neurology, Boston University School of Medicine and the Boston Veterans Administration Hospital, Boston, Massachusetts".

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

A Guide to the Use of Terms in Plant Patholog Prepared by The Terminology Sub-Committee c the Federation of British Plant Pathologists. (Ph topathological Papers, No. 17.) Pp. iv + 54. (Kew Surrey: Commonwealth Mycological Institute, 1973.) £1.30. [2611

Principles of Free Radical Chemistry. By Dr J. I. G. Cadogan. (The Chemical Society Monographs for Teachers, No. 24.) Pp. 83. (London: The Chemical Society, 1973.) £1. [2811

The British Council. Annual Report, 1972/73. Pp. 102. (London: The British Council, 1973.) [2811

The Royal Society. Report of Council for the year ended 31 August, 1973. Pp. 109. (London: The Royal Society, 1973.) [2911

Research Supported by the Social Science Research Council 1973. Pp. 318. (London: Social Science Research Council, State House, High Holborn, 1973.) 75p net. [2911

Inter-Research Council Committee on Pollution Research. Cadmium in the Environment. (Report of a Seminar held at Alhambra House, Charing Cross Road on 15 March, 1973.) Pp. 39. (London: Natural Environment Research Council, 1973.) [3011

Other Countries

Teoria Generale del Lavoro Umano, Volume Primo. By Pier Sergio. Vassallo. Pp. 101. (Milano: Pier Sergio Vassallo, c/o CFM-Via Clerici 10, 1973.) [2611

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Tropical Agronomy, 1972/1973. Pp. 128. (Brisbane: CSIRO, 1973.) [2611

Dynapol: Forging Safer Links in the Food Chain. Pp. 16. (Palo Alto, California: Dynapol, 1454 Page Mill Road, 1973.) [2611

Republic of South Africa: Department of Industries. Sea Fisheries Branch Investigational Report No. 101: Growth of the South African Maasbanker *Trachurus trachurus* Linnaeis amd Age Composition of the Catches 1950–1971. By N. D. Geldenhuys. Pp. 24. (Sea Point, Cape Town: Sea Fisheries Branch, Beach Road, 1973.) [2711

Australia: Commonwealth Scientific and Industrial Research Organization. Twenty-fifth Annual Report, 1972/1973. Pp. 99. (Melbourne: CSIRO, 1973.) [2811

Republic of South Africa: Department of Industries. Division of Sea Fisheries Investigational Report No. 98: Marine Alpha-Radioactivity Off South Africa. 1. Gross Alpha-Activity, Radiation Dose, Alpha-Spectrum and Variations in Alpha-Activity of Marine Life. By L. V. Shannon. Pp. 80. (Sea Point, Cape Town: Division of Sea Fisheries, Beach Road, 1973.) [2811

Our Agricultural Future. By Dr M. S. Swaminathan. (Sardar Patel Memorial Lectures, 1973, at India International Centre, New Delhi). Pp. 54. (New Delhi: All India Radio, 1973.) [2811

Priorities for Action: Final Report of the Carnegie Commission on Higher Education, with Technical Notes and Appendixes. Pp. x + 243. (New York and London: McGraw-Hill Book Company, 1973.) $4.95. [2811

Nuclear Energy Agency, OECD. Radiation Protection Standards for Gaseous Tritium Light Devices. Pp. 23. (Paris: Nuclear Energy Agency, OECD, 1973.) [2811

Republic of Cyprus: Ministry of Agriculture and Natural Resources. Annual Report of the Geological Survey Department for the year 1972. By Y. Hadjistavrinou. Pp. 33. (Nicosia: Geological Survey Department, Ministry of Agriculture and Natural Resources, 1973.) [2811

OECD. Nuclear Energy Agency. Thirteenth Annual Report of the ORCD Halden Reactor Project 1972. Pp. 178. (Paris: OECD, Nuclear Energy Agency, 1973.) [2811

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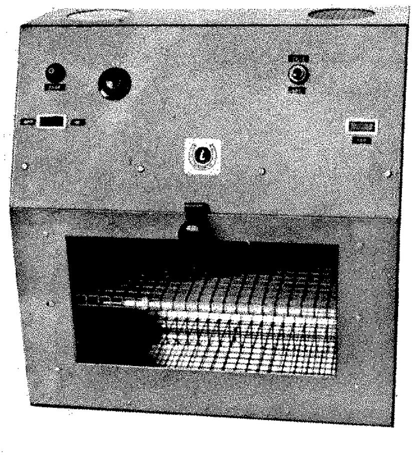

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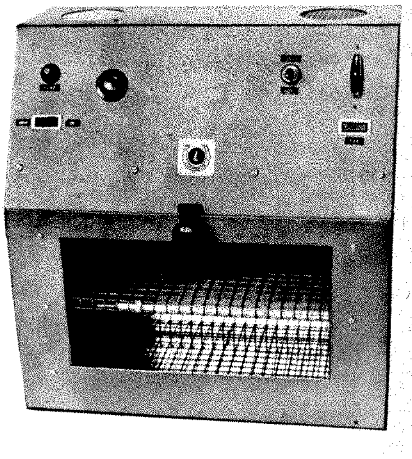

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Applications are invited for a position of Lecturer in the Statistics Section of the Mathematics Department.

The Statistics Section has 15 members of staff whose interests include aspects of stochastic processes, multivariate analysis, foundations of statistical inference, mathematical genetics, decision theory, measure theory. The section is also the focus of a biomathematics programme.

Salary scale: $A8,698 to $A11,982 per annum with superannuation based on an endowment assurance scheme, the employee and employer contributing 5 per cent and 10 per cent respectively.

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Further general information and details of the plication procedure are available from the Academic egistrar, Monash University, Wellington Road, Clayton, Victoria, 3168 Australia, or the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF. Enquiries about the Department may be directed to Professor J. S. Maritz, in the University. Applicants should quote reference no. 40112.

Closing date: **January 31, 1974.**

The University reserves the right to make no appointment or to appoint by invitation.

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3. JUNIOR TECHNICIAN

Required in Clinical Immunology Laboratory interested in the mechanisms of inflammation. Previous experience of immunological techniques an advantage. Qualifications: "O" level passes in English, Maths and 2 Science subjects. Salary in range £840 to £1,575 per annum according to age and qualifications. Ref. 2/143/N.

4. JUNIOR TECHNICIAN/TECHNICIAN

Required for research work on venous thrombosis and rheumatoid arthritis. Experience of haematological techniques and animal work an advantage. Minimum qualifications "O" level passes in English, Maths and 2 Science subjects. (Salary scale £840 to £1,575 per annum.) Candidates with AIMLT/HNC will be graded as Technician (£1,578 to £1,794 per annum). Ref. 3/226/N.

Applications to the Secretary, R.P.M.S., Hammersmith Hospital, DuCane Road, London W12 0HS, quoting the appropriate reference. (229)

ICI Pharmaceuticals Division

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M. F. Losse, Personnel Officer,
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GOVERNMENT OF HONG KONG

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(221)

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☐ Application forms from Ministry of Defence (Procurement Executive) SPM1b3 Room 402, Savoy Hill House, Savoy Hill, London WC2R 0BX, telephone 01-836 1207 ext. 1667/1665.

British Museum
Research Laboratory, London

Chemist

■ To examine and develop conservation methods for a wide range of organic objects, particularly library and archival material.
☐ 1st/2nd hons degree or equivalent in Chemistry ☐ At least 2 years' research experience ☐ Interest in bio-deterioration an advantage ☐ Age normally under 30 ☐ Appointment as Higher Scientific Officer (£2251-£2842) ☐ Ref: SB/3/DJA ☐ Closing date 1 February 1974.
☐ Application forms from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

British Museum (Natural History)

Zoologist

■ Curatorial and advisory work in Fish Section ■ Opportunity for taxonomic research.
☐ Degree/HNC or equivalent in Zoology ☐ Age under 30 ☐ Appointment as Higher Scientific Officer (£2251-£2842) or Scientific Officer (£1493-£2352) according to age and experience ☐ Ref: SB/15/DK ☐ Closing date 1 February 1974.
☐ Application forms from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Ministry of Agriculture, Fisheries and Food
Pest Infestation Control Laboratory, Surrey

Zoologist

■ In Land Pests and Bird Research Department ■ To join team studying the biology and control of harmful birds ■ Initial work on starlings ■ Assist with projects on other species ■ Extensive field work and travel involved.
☐ 1st/2nd hons degree or equivalent in Zoology ☐ At least 2 years' postgraduate experience ☐ Driving ability essential ☐ Age normally under 32 ☐ Appointment as Senior Scientific Officer (£2615-£3640) or Higher Scientific Officer (£2076-£2667) ☐ Ref: SB/6/AF ☐ Closing date 1 February 1974.
☐ Application forms from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext 500 or London 01-839 1992 (24 hour answering service.)

Ministry of Agriculture, Fisheries and Food
Fisheries Experiment Station, Conway, Caerns

Botanist/ Microbiologist

■ In Shellfish Culture Unit ■ Operate existing algal culture systems ■ Investigate food value of novel and existing algae ■ Devise new techniques for large scale culture.
☐ Degree/HNC or equivalent in Botany or Microbiology ☐ Mechanical aptitude an advantage ☐ Age under 27 ☐ Appointment as Scientific Officer (£1318-£2177) ☐ Ref: SB/1/A ☐ Closing date 1 February 1974.
☐ Application forms from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Centre for Overseas Pest Research
London

Plant Diseases Editor (Pans)

■ To work on quarterly journal dealing with tropical crop protection ■ Commission articles from authors ■ Attend Conferences, Meetings and prepare reports ■ Visit commercial companies and research stations for developments in the field.
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☐ Application forms from Administrative Secretary, Centre for Overseas Pest Research, College House, Wrights Lane, London, W8 5SJ.

Ministry of Agriculture, Fisheries and Food
Salmon and Freshwater Fisheries Laboratory,
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■ To join team assessing potential of grass carp for aquatic weed control ■ Help develop new laboratory technique to examine effects of adverse conditions on fish behaviour ■ Field work and variety of other duties involved.
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Science group
CIVIL SERVICE

(186)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF CHEMISTRY

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Applications are requested for the post of Postdoctoral Research Assistant who will work under the joint direction of Professor E. A. V. Ebsworth and Dr T. A. Stephenson on isomerisation and exchange in some compounds of selenium. The work will involve synthetic and spectroscopic studies, and applicants should have had experience in the handling of compounds that are thermally unstable and sensitive to air and moisture.

The contract will run for 2 years at a starting salary of up to £2,058, starting on April 1, 1974.

Applications (in writing) should be addressed to: Professor E. A. V. Ebsworth, Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ.

The closing date for applications is March 1, 1974. Please quote reference 5000. (206)

UNIVERSITY
OF DUBLIN
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The Staff Secretary,
West Theatre,
Trinity College,
Dublin 2,

who will receive completed applications up to February 18, 1974.

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UNIVERSITY OF OXFORD

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Applications (if possible, seven copies, except in the case of overseas applicants who need only submit one copy) should be sent to Professor J. W. S. Pringle, Department of Zoology, South Parks Road, Oxford OX1 3PS, not later than February 28. They should include a statement of age, qualifications and experience, and the names of two referees. (106)

DEPARTMENT OF BIOLOGY
QUEEN'S UNIVERSITY
KINGSTON, ONTARIO

Applications are invited for 3 positions in this department in the general areas (i) vertebrate biology, (ii) cell or developmental biology, and (iii) experimental plant science. Well qualified candidates in related fields may also be considered.

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The Secretary,
Health Education Council,
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London WCIA IAH.

The
Health
Education
Council

(189)

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This is a unique post in scientific, technical and medical publishing. The creative planner will work closely with our house editors to report on progressive topics in biological, medical and social sciences, for which useful and marketable titles should be commissioned; this will entail extensive discussions with experts in Universities and Industries. The creative planner will equally be responsible for the promotion and advertising of a very broad range of new and backlist titles under the broad heading of Life Sciences. This work will entail the assessment of the market (world-wide); the preparation of suitable 'communication' material which will be readily acceptable and of interest to scientists; liaison with sales and editorial staff and the ability to continually assess performance and plan work in advance. This will be a particularly fruitful job to anyone who wishes to work across a broad spectrum.

The applicant must be prepared to travel and should preferably be able to drive. Write giving full details to:

Training & Personnel Manager,
Pergamon Press Ltd, Headington Hill Hall, Oxford OX3 0BW (235)

DUBLIN INSTITUTE FOR ADVANCED STUDIES

SCHOOL OF THEORETICAL PHYSICS

Applications are invited for a post as Assistant Professor in the School tenable for one or two academic years commencing October 1, 1974.

Salary: £3,091 per annum plus marriage and family allowances.

Applicants should send a curriculum vitae and list of publications, together with the names of two referees, so as to reach the undersigned not later than March 15, 1974.

The Registrar,
Dublin Institute for Advanced Studies,
10 Burlington Road,
Dublin 4, Ireland. (160)

CSIRO

AUSTRALIA

RESEARCH SCIENTIST

DIVISION OF MINERALOGY-FLOREAT PARK, W.A.

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 or whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD: GEOLOGY

GENERAL: The Division of Mineralogy, a constituent of the Minerals Research Laboratories, conducts research on the mode of origin and characterization of deposits of economic minerals, and on the development of techniques in exploration. It has its headquarters in Floreat Park, W.A. and laboratories at North Ryde, N.S.W. and Canberra, A.C.T. The Appointee will be located at Floreat Park. As part of its geochemical investigations the Division is collaborating with the Division of Soils on the inter-relation of secondary geochemical dispersion, regional structural patterns, geomorphology and paleography, particularly in the arid areas of the Archaean Shield of Western Australia.

DUTIES: A senior research geologist is required to take part in this programme, initially aiming to elucidate the development of the present landscape through geological time, and the consequences of this development in determining geochemical dispersion.

QUALIFICATIONS: A Ph.D. in geology and demonstrable research ability. Appropriate research experience preferably with an established interest in the paleogeography of arid terrains. Experience of the Yilgarn Block of Western Australia would be a special advantage.

SALARY: Appointment will be made within the salary ranges of Senior Research Scientist or Principal Research Scientist $A12,298–$A17,077 p.a.

TENURE: This is a three year appointment.

Applications stating full personal and professional details, the names of at least two professional referees and quoting reference number 604/114 should reach:

The Personnel Officer,
Australian Scientific Liaison Office,
64–78 Kingsway,
London,
WC2B 6BD
by the 15th February, 1974

Applications in U.S.A. and Canada should be sent to
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
Washington, D.C. 20036, U.S.A.

(227)

AGRICULTURAL RESEARCH COUNCIL

MEAT RESEARCH INSTITUTE

Applications are invited for a new post in the Abattoir Section which will be concerned initially with investigations into stress and disease transmission during transport and lairing of slaughter stock in relation to carcass quality.

MINIMUM QUALIFICATIONS: A first or upper second class honours degree in animal science or physiology, together with appropriate postgraduate research experience.

SALARY: Scientific Officer scale (£1,318-£2,177 p.a.). Higher Scientific Officer scale (2,076-£2,667 p.a.) depending on qualifications and experience; a minimum of 2 years postgraduate experience being required for appointment in the HSO grade. A higher grading may be offered to a particularly well qualified candidate.

This post is permanent and pensionable (FSSU with a 4½% allowance on salary to offset contributions).

Application forms quoting AB 18: Secretary, Meat Research Institute, Langford, Bristol, BS18 7DY. (201)

UNIVERSITY OF GUELPH

DEPARTMENT OF CHEMISTRY

FACULTY POSITION

Applications are invited for a faculty position at the rank of professor, to begin September 1, 1974, to January 1, 1975. Candidates must have a Ph.D. degree in chemistry or biochemistry, a considerable record of research accomplishment in biochemistry or biophysical chemistry, and teaching experience in chemistry or biochemistry. Salary will depend on qualifications and experience. Duties include teaching, supervision of graduate research, and independent research.

Applicants should provide a full curriculum vitae and the names of three referees to A. K. Colter, Chairman, Department of Chemistry, University of Guelph, Guelph, Ontario N1G 2W1, Canada. (115)

TECHNICIAN required to assist with original biochemical and immunological research on cancer. Experience in a relevant subject is desirable but not essential. The successful candidate will be appointed on a salary scale of £1,440 to £2,292.—Apply enclosing a curriculum vitae and the names of two referees to: Dr. G. Wiernik, Research Institute, Churchill Hospital, Headington, Oxford. (91)

THE CHINESE UNIVERSITY OF HONG KONG

Applications are invited for the following positions in the University. The Colleges to which posts are assigned are specified in brackets.

POST AND SPECIALISATION

FACULTY OF SCIENCE

SENIOR LECTURESHIP IN BIOCHEMISTRY (United College): The candidate should possess a Ph.D. degree or its equivalent with expertise in the field of Endocrinology. Preference would be given to candidates who are also experienced in Biochemical Embryology and related areas.

LECTURESHIP IN BIOCHEMISTRY (United College): The candidate should have a Ph.D. degree or its equivalent with training in Human Biochemistry and/or Nutrition. Prior teaching experience is essential. Demonstrable ability in research will a definite asset.

LECTURESHIP IN BIOLOGY (Chung Chi College): Applicants should hold a higher degree or have good experience in Marine Biology; experience of mariculture would be an advantage. The appointee will take part in the teaching of the marine sciences and may be required also to undertake teaching in the more general courses provided by the department. There are excellent facilities at the Marine Science Laboratory of the University for teaching and research.

GENERAL REQUIREMENTS

Candidates for Senior Lectureships should have a higher degree, preferably a Ph.D. in a relevant field, considerable university teaching and research experience and academic publications of originality and merit. Candidates for Lectureships should have a higher degree (preferably a Ph.D.) in a relevant field, with appropriate university teaching and/or research experience.

SALARY (IN £)

Senior Lecturer: £5,797 to £7,787 per annum (man or woman); Lecturer: £3,720 to £6,234 per annum (male), £3,574 to £6,234 per annum (female).

(Entry points depend on qualifications and experience. Equalisation of male and female salaries will be achieved by April 1975.)

OTHER CONDITIONS OF SERVICE

These include: (1) Superannuation (5 per cent employee, 15 per cent University); (2) Medical benefits; (3) Long leave (for all appointees except Language Instructors); (4) Housing accommodation or allowance in lieu thereof for all Senior Lecturers and appointees on Lecturer rank engaged on overseas terms. Housing allowance for appointees at Lecturer rank on local terms whose salary is above the Bar (married women excepted); (5) Passages for appointees on Assisted Lecturer rank and above recruited from abroad on appointment, and also on home leave if engaged on overseas terms.

ASSUMPTION OF DUTY

All appointees are expected to assume duty August 1, 1974, or as soon as possible thereafter.

APPLICATION PROCEDURE

Further details and application forms may be obtained from the Registrar/Academic Registrar of the College concerned. All three Colleges are situated at Shatin, N.T. Application forms, in triplicate, should be returned together with a covering letter and copies of credentials, marked "APPLICATION" on cover, as soon as possible. (199)

PAHLAVI UNIVERSITY

COLLEGE OF ARTS AND SCIENCES
SHIRAZ, IRAN

Applications are invited for positions in Astronomy and Physics beginning fall 1974, at the ASSISTANT/ASSOCIATE PROFESSOR level. Duties include teaching B.Sc. and M.Sc. students and some research. Field of solid state, nuclear, and astrophysics are represented in the department. A Varian X-band E.P.R. Spectrometer is in operation, and a 20-inch Cassegrain reflector with a photoelectric photometer is expected to be in operation by July 1974.

Ph.D. is required. Appointments will be made on a two-year renewable contract basis with an annual salary range equivalent to $12,000 to $16,000 depending on qualifications and experience. Return fare included.

Send résumé, curriculum vitae and confidential recommendations from three references to Dean Vessal, from whom particulars may be obtained. Deadline for applications is April 1, 1974. (107)

THE QUEEN'S UNIVERSITY OF BELFAST

CHAIR OF GEOLOGY

Applications are invited for the Chair of Geology which will be vacated on September 30, 1974, on the resignation of Professor Alwyn Williams, FRS. The salary is £6,462 with contributory pension rights under the FSSU.

Applications should be received by March 1, 1974. Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland. (Please quote Ref. 74/N.) (207)

UNIVERSITY OF PRETORIA, PRETORIA, SOUTH AFRICA

INSTITUTE FOR GEOLOGICAL RESEARCH ON THE BUSHVELD COMPLEX

DIRECTOR

Applications are invited for the post of Director of the above-mentioned Institute which is to be established and attached to the Department of Geology. The Director will have full professorial status and as chief executive officer of the Institute will be assisted by a Board whose members represent organisations subventing the Institute. His duties will include the following:

(a) To execute the research policy of the Board of Control.

(b) To coordinate and guide research on the Bushveld Complex by research scholars and postgraduate students.

(c) To give a limited number of lectures to postgraduate students in the Department of Geology.

Candidates must have at least a Ph.D. or equivalent qualification.

Salary: R7,500 × 300–R9,900 plus an additional 15% of salary in the form of a pensionable allowance (£1 = R1,60 approximately). The commencing salary will depend upon qualifications and experience.

A vacation bonus is payable according to Public Service regulations. The University has a pension fund, a medical aid fund, a subsidy scheme for housing loans and a group insurance policy scheme with attractive benefits, for permanent full-time members of staff.

Application forms are obtainable from the Registrar (Academic), University of Pretoria, Pretoria 0002, South Africa.

Applications close on February 28th, 1974.

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BLUE CIRCLE GROUP

inorganic chemist - kent

Current demand for cement and cement products enables this prominent company to provide opportunity which few others can match. This position carries an excellent negotiable salary and other benefits, which include profit sharing bonus, pension and advantageous house purchasing schemes, after appropriate qualifying service.

This is an ideal opportunity for a young graduate to gain invaluable experience. You will be involved in Research and Development work concerning special cements as well as cement based materials and associated products.

Although some post graduate experience in silicate chemistry or cement based materials would be advantageous, it is not essential.

Please include full professional details in your letter to:

The General Manager,
The Associated Portland Cement
Manufacturers Ltd,
Central Research Laboratories,
Greenhithe, Kent.

(222)

THE OPEN UNIVERSITY

ACADEMIC APPOINTMENTS IN EARTH SCIENCES AND CHEMISTRY

EARTH SCIENCES

Two appointments are available, either as two-year contract Lectureships or as one/two-year Secondments. Successful candidates will assist in the preparation of a third level course in Earth Science Techniques and Methods.

CHEMISTRY

Two appointments are available. One appointment will be made with tenure; the second appointment will be a two-year contract Lectureship or one/two-year Secondment. Candidates should have special interests in either Organic Chemistry or in Inorganic/Physical Chemistry.

The preparation of Open University courses involves writing course text and assessment materials, making television and radio programmes, devising home experiment kits, and planning and participating in Summer School activities. Applicants should have a keen interest in teaching and should be willing to experiment with the media available. The successful candidates will be expected to maintain an active interest in their research field.

Appointments are available from April 1, 1974, or as soon as possible thereafter. Lecturer salary scale: £1,929-£4,548 per annum with FSSU benefits.

Full details are available from The Acting Personnel Manager (SL3), The Open University, P.O. Box 75, Walton Hall, Milton Keynes, MK7 6AL. (Tel: Milton Keynes 74066, ext. 3068.) Closing date for applications: Thursday, February 7, 1974. (200)

CSIRO

AUSTRALIA

RESEARCH SCIENTIST

AGRO-INDUSTRIAL RESEARCH UNIT—
CANBERRA, A.C.T.

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD: **SIMULATION OF AGRO-INDUSTRIAL SYSTEMS**

GENERAL: The newly created Agro-industrial Research Unit (of three scientists) will examine the possibility of exploiting the year-round growth of crops in the tropics by year-round harvesting and industrial processing of a range of crops, and by feeding of cattle on industrial by-products. Related studies in crop processing and animal feeding are being undertaken at the CSIRO Divisions of Applied Chemistry in Melbourne and Animal Physiology in Sydney respectively.

The appointee will be based in Canberra, but will travel extensively to collaborating laboratories in Sydney and Melbourne and to Northern Australia.

DUTIES: To undertake collaborative research into simulation of the biological, physical and economic processes of complex agro-industrial systems.

QUALIFICATIONS: A Ph.D. degree or equivalent qualifications in science, agricultural science or engineering, and demonstrable research ability. Some experience in simulation and economics is essential.

SALARY: The appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: $A9,380–$A14,290 p.a.

TENURE: Either an indefinite or a fixed term appointment may be negotiated. An indefinite appointment carries Australian Government Superannuation benefits.

Applications stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number 267/5, should reach:

The Personnel Officer,
Australian Scientific Liaison Office,
64–78 Kingsway,
London,
WC2B 6BD
by the 15th February, 1974

Applications in U.S.A. and Canada should be sent to
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
Washington, D.C. 20036, U.S.A.

(225)

UNIVERSITY OF LONDON
INSTITUTE OF PSYCHIATRY
DEPARTMENT OF NEUROLOGY

RESEARCH WORKER required immediately for biochemical/physiological studies in experimental epilepsy. Neurochemical experience an advantage. Applicants wishing to visit the Laboratory may phone 01-703 5411, ext. 370, for an appointment. Initial salary up to £1,929 plus £162 London allowance, renewable annually up to 3 years.

Apply to the Secretary, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, quoting reference BSM/N. Applications should be received by January 29, 1974. (183)

UNIVERSITY OF KEELE
LECTURER IN MATHEMATICS

Applications invited for post of Lecturer in Mathematics. Initial salary on points 1 to 3 of scale £1,929-£4,548 per annum. Further particulars and application forms from The Registrar, The University, Keele, Staffs., ST5 5BG, to whom they should be returned by February 1, 1974. (219)

SWISS INSTITUTE OF TECHNOLOGY
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a junior and senior research position in the field of membrane biochemistry. Candidates for the junior position should have recently obtained their Ph.D. For the senior position candidates should have at least two years post-doctoral research experience.

Applications should be sent to: Professor G. Semenza, Swiss Institute of Technology (ETH), Universitätsstr. 16, CH-8006 Zürich, Switzerland. (216)

UNIVERSITY OF CAMBRIDGE
RESEARCH ASSISTANT

Applications are invited for the position of Graduate Research Assistant from February 1 to July 30, 1974. The work will involve the application of operant techniques to the study of nest-building behaviour in canaries.

Applications including a brief curriculum vitae and the names of two referees should be sent to Dr Joan Stevenson-Hinde, Sub-Department of Animal Behaviour, Madingley, Cambridge CB3 8AA. (110)

UNIVERSITY OF NEW SOUTH WALES
SCHOOL OF ANATOMY
LECTURER

Distinguished academic record required. Medical qualifications advantageous. Facilities exist for wide range of research including electron microscopy, qualitative and quantitative cytology and tissue culture.

Salary $A8,698 range $A11,982 per annum. Commencing salary according to qualifications and experience.

For details of appointment, including superannuation, study leave and housing scheme, write to the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close in Australia and London on **March 1, 1974.** (230)

THE UNIVERSITY OF MANCHESTER
LECTURER IN PSYCHOLOGY

Applications are invited from candidates with special competence in the sphere of child study. The successful candidate, who will succeed Dr R. R. Skemp on his appointment to a Chair at the University of Warwick, will direct the Child Study units in the Department. Salary range per annum: £1,929 to £4,548. F.S.S.U.

Further particulars and application forms (returnable by February 1) from the Registrar, The University, Manchester M13 9PL. Quote ref.: 1/74/Na (233)

THE UNIVERSITY OF MANCHESTER
LECTURER IN ZOOLOGY

Applications invited for the above post. An interest in ecology, entomology or chordate biology would be an advantage. Salary scale per annum: £1,929 to £4,548, but initial salary will not exceed £2,388 per annum. F.S.S.U.

Particulars and application forms (returnable by February 23) from the Registrar, The University, Manchester M13 9PL. Quote ref.: 5/74/Na. (234)

UNIVERSITY OF ALBERTA

Applications for GRADUATE STUDIES IN PHARMACOLOGY for the degrees of M.Sc. and Ph.D. are invited from students with a background in Biochemistry, Pharmacy, Biology, Chemistry or Medicine. Opportunities exist in Biochemical Pharmacology, Neuropharmacology and Smooth Muscle Pharmacology. Successful applicants may be eligible for financial assistance.

For further information write to: Dr J. S. Charnock, Chairman, Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada, T6[illegible] 2H7. (X3)

UNIVERSITY OF ALBERTA

Applications are invited for a post-doctoral position in Biochemistry available in the summer of 1974 for two years. The areas of research include DNA replication, circular DNAs and multistranded polynucleotides. Candidates should have a strong chemical and physical background. A Ph.D in chemistry would be a suitable candidate. The starting salary would be $8,900 p.a.

A curriculum vitae and letters of recommendation are required from applicants who should write to Dr A. R. Morgan, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2E1. (211)

WESTFIELD COLLEGE
(UNIVERSITY OF LONDON)
HAMPSTEAD, NW3 7ST

GRADUATE EXPERIMENTAL OFFICERS required in the Chemistry Department for work in connection with the Circular Dichroism Unit, and the Medical Research Council Steroid Reference Collection. Salary scale £1,679 to £1,939 per annum according to age and experience.

Please write or telephone to the Personnel Officer (N), Westfield College, Hampstead NW3 7ST. 01-435 7141. (197)

GRADUATE RESEARCH ASSISTANT REquired for research project in human pathology. Degree in biology and experience in electron microscopy technology preferred. Salary in region of £1,600.—Applications to Professor W. B. Robertson, Histopathology Department, St. George's Hospital, Blackshaw Road, Tooting, London SW17 0QT. (185)

APPLICATIONS ARE INVITED FOR AN appointment involving undergraduate instruction in **mineralogy** and **petrography** and interaction with the graduate programme. The Department's research activity emphasizes environmental geology and preference will be given to applicants concerned with the mineralogy and geochemistry of sedimentary environments. Rank open; salary commensurate with experience. A temporary one-year appointment is also available in **economic geology.**—Send résumé to: Dr R. N. Farvolden, Chairman, Department of Earth Sciences, University of Waterloo, Waterloo, Ontario, N2L 3G1. (210)

THE QUEEN'S UNIVERSITY
OF BELFAST

LECTURESHIP IN PHYSICS

(Optical and Magneto-Optical Properties of Thin Films)

Applications are invited for the post of Lecturer in Physics, tenable from October 1, 1974, or such other date as may be arranged. The successful applicant will be required to undertake teaching at undergraduate and postgraduate level and to take part in research within a group (under the general direction of Professor P. H. Lissberger) concerned with studies of the optical and magneto-optical properties of thin solid films in relation to their structure. Preference will be given to applicants with experimental and theoretical interests in this field. Previous experience of the measurement and interpretation of the optical constants of solids is desirable. The appointment will be subject to a period of probation of up to three years in duration. Initial placing which will depend on experience and qualifications will be made at one of the first three points on the scale for lecturers: £1,929, £2,058, £2,223, rising to £4,548 with contributory pension rights under the FSSU.

Applications should be received by February 28, 1974. Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. (Please quote Ref. No. 74/N.) (208)

PLYMOUTH GENERAL HOSPITAL

A SCIENCE GRADUATE is required to fill a new post based at Plymouth General Hospital, for work principally involving saturation analysis and related techniques. Initially it is intended to set up tests of pituitary, thyroid and adrenal function, plus a number of other assays, and there will be some work involving liquid scintillation counting. In addition to the laboratory techniques, the successful candidate will take day-to-day charge of automatic counting equipment.

Previous experience in this field of work is sential and further details may be obtained by ntacting Dr. Janice Went, telephone Plymouth --003.

Application forms may be obtained from the Hospital Secretary, Plymouth General Hospital, North Friary House, Plymouth PL4 8QQ, and should be returned by not later than 14 days from the appearance of this advertisement. (191)

UNIVERSITY OF ABERDEEN
DEPARTMENT OF MEDICAL PHYSICS

M.Sc. IN MEDICAL PHYSICS

The Department provides a 12 months' course in Medical Physics, commencing October 1 each year. Candidates should have a first or second-class honours degree in Physics or Electronics or Engineering, or equivalent qualifications.

The course covers a wide range of the applications of physics to medicine and is designed to prepare candidates for a career in Medical Physics.

The Science Research Council and Medical Research Council have accepted the course as suitable for the tenure of their Advanced Course Studentships. The course has been endorsed by the Departments of Health in London and Edinburgh.

Further details available from the Secretary, Department of Medical Physics, University of Aberdeen. (X77)

MASSEY UNIVERSITY
PALMERSTON NORTH, NEW ZEALAND

LECTURER IN GENETICS

Applications are invited for the above-mentioned position in the Department of Microbiology and Genetics; the department is responsible for courses leading to B.Sc., B.Sc.(Hons.), M.Sc. and Ph.D. degrees. The successful applicant will assist in the further development of Genetics as an area of teaching and research and will participate in the offering of an introductory course in cell biology to first year students.

Salary: NZ$6,575 to $NZ8,343.

Further details of this position and of the University, together with general conditions of appointment, may be obtained from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on **February 15, 1974.** (195)

Ministry of Agriculture, Fisheries and Food
Plant Pathology Laboratory, Harpenden, Herts.

Agricultural Entomologist

■ **To join team providing technical advice on maintenance of high health standards in consignments of imported/exported plants and plant produce ■ Liaison with Plant Health and Seeds Inspectorate and assistance with technical training in entomology.**
☐ Degree or equivalent in Applied Biology, Agriculture or Horticulture ☐ Age under 30 ☐ Appointment as Higher Scientific Officer (£2076-£2667) or Scientific Officer (£1318-£2177) according to age and experience ☐ Ref: SB/1/AG ☐ Closing date 1 February 1974.
☐ Application forms from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Department of Health and Social Security, London

Information Scientist

■ **In Medical Division ■ To compile information on chemical contamination of food, and environmental pollution ■ Help develop data storage and retrieval system.**
☐ Degree, HNC or equivalent in chemical or biological subject ☐ Age under 27 ☐ Appointment as Scientific Officer (£1493-£2352) ☐ Ref: SB/1/K ☐ Closing date 1 February 1974.
☐ Application forms from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Department of the Environment
Water Pollution Research Laboratory

Chemist/Chemical Oceanographer

■ **Develop methods to predict effects of polluting substances in estuaries ■ Undertake studies of degradation of organic matter ■ Field studies on solution rate of oxygen through air/water interface.**
☐ Degree/HNC or equivalent in Chemistry of Chemical Oceanography ☐ Age under 27 ☐ Appointment as Scientific Officer (£1318-£2177) ☐ Ref: SA/10/HE ☐ Closing date 1 February 1974.
☐ Application forms from The Director, Water Pollution Research Laboratory, Elder Way, Stevenage, Herts, SG1 1TH.

Department of the Environment
Building Research Station
Princes Risborough Laboratory

Chemist/Physicist

■ **To study physical properties of protective surface coatings of wood-based panel products ■ Development of predictive performance tests.**
☐ Degree/HNC or equivalent in Chemistry, Physics or Materials Science ☐ Experience in applying physical techniques in surface coating or polymer technology an advantage ☐ Age under 30 ☐ Appointment as Higher Scientific Officer (£2076-£2667) or Scientific Officer (£1318-£2177) according to age and experience ☐ Ref: SA/37/HA ☐ Closing date 1 February 1974.
☐ Application forms from The Secretary, Princes Risborough Laboratory, Princes Risborough, Aylesbury, Bucks, HP17 9PX.

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Science group
CIVIL SERVICE

UNIVERSITY OF CALGARY, GRADUATE Teaching and Research Assistantships are available for students proceeding to the Ph.D. or M.Sc. degrees in all branches of Chemistry and Biochemistry. The Assistantships are valued from $4,100 to $5,000 per annum and carry remission of programme fees and travel allowance.

For further information write to: Department of Chemistry, Calgary, Alberta T2N 1N4, Canada. Please indicate field of interest. (X35)

RESEARCH TECHNICIAN required for work on the development of assays for vitamin-D and related compounds. Experience of radioimmunoassays and competitive protein binding assays useful but not essential. Salary on the Technician Whitley Council scale according to experience.—Applications, together with qualifications and names of two referees to Dr J. L. H. O'Riordan, Medical Unit (N), The Middlesex Hospital Medical School, London, W.1. Telephone 636 8333 Ext. 595. (232)

Senior Pharmacologist for Research

Applications are invited for a senior post in our Pharmacology Department in Welwyn Garden City. The Department is responsible for carrying out research and screening work aimed at the development of new drugs and the pharmacologists participate in the research programmes of interdisciplinary groups working in specific areas.

The senior pharmacologist we appoint will direct the research programme of a team of pharmacologists engaged in cardiovascular research. Applicants should be in their thirties and hold a Ph.D., together with at least five years postdoctoral experience. Previous experience in cardiovascular research is desirable but not essential and of less importance than proven ability to direct original and creative research.

Our Pharmacology Department has recently moved into new laboratories where the facilities and working conditions are excellent. Conditions of Service will be attractive and in appropriate cases assistance with relocation will be offered.

Roche Products Limited is part of a major international pharmaceutical concern based in Switzerland and is itself one of the leaders in the industry in the United Kingdom. Applications in writing should be addressed to the Senior Welwyn Personnel Officer.

ROCHE

Roche Products Limited
Welwyn Garden City Hertfordshire AL7 3AY

(194)

Head of Animal Supplies

The Research Department, situated in Nottingham, has a vacancy for a person to take full charge of the Animal Supplies and Services Unit, with the responsibility for the breeding, purchase, handling and health of a wide variety of laboratory animals for use in medical research. The Unit, comprising small animal, dog and primate sections, is being enlarged. Extensive new animal facilities are planned and the man appointed will be expected to participate in the development of this project. Staff numbers over 25.

The person appointed is likely to be qualified in veterinary science and may possess experience of laboratory animal husbandry.

Employment conditions are attractive and include profit sharing and contributory pension schemes. Assistance with re-location expenses will be given in appropriate cases.

Please write for further details and application form to: J. F. Pattison, Employment Manager,

Boots

The Boots Company Ltd.,
Station Street, Nottingham NG2 3AA.

(159)

UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

TEMPORARY LECTURESHIP IN GENETICS

A temporary lectureship in Genetics for one year, with possible extension for a second year, is immediately available.

Teaching responsibilities will be primarily for a second-year class, and will cover aspects of Metazoan Genetics.

The research interests of the associated group is in the developmental genetics of *Drosophila*, including Embyrology and cell culture and good facilities are available. Candidates with other research interests will also be considered.

Appointment will be made on the Lecturer scale: £1,929 to £4,548 per annum, as soon as possible.

Application forms may be obtained from the Secretary of Science, Science Office (E), University of Sussex, Falmer, Brighton BN1 9RH.

(224)

BAKER MEDICAL RESEARCH INSTITUTE

MELBOURNE, AUSTRALIA

(RE-ADVERTISEMENT)

The Trustees of the Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute invite applications for the position of:

DIRECTOR OF THE INSTITUTE

The present Director will be retiring from appointment in December 1974.

THE INSTITUTE

The Baker Institute is an autonomous organisation situated in the grounds of Alfred Hospital and adjacent to the Clinical Sciences Building of Monash University Medical School. The Institute is affiliated with Monash University which means that the research facilities of the Institute are available for promoting the teaching of both undergraduate and postgraduate students of the University, up to Ph.D. level. For both bodies it makes possible the integration of trained personnel and the research facilities of both the University and the Institute, for the benefit of medical education and research. The main financial support of the Institute is presently derived from the Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions.

The Institute building has recently been rebuilt, all laboratories are air-conditioned and equipped for work in biochemistry, physiology, pharmacology and histology. The laboratories of Alfred Hospital Clinical Research Unit are presently housed in the Institute building. The building is linked with the new Alfred Hospital Ward Block now under construction. Arrangements with the Hospital will permit the successful applicant access to clinical facilities, including beds, if such are appropriate to the research programme.

REQUIREMENTS

Applicants must be medical or biological scientists with research experience, demonstrated ability in team leadership and some appropriate administrative experience.

CONDITIONS

* The appointment is for at least five years and will be reviewed not later than four years after the appointment, when it may be extended as appropriate.
* A generous salary is contemplated, and will be based on qualifications and experience.
* Superannuation is available under Institute Scheme.
* Commencing date January 1975 or later by negotiation.
* Assistance will be given in meeting the cost of removal expenses to a successful applicant not resident in Melbourne and in some circumstances with the cost of repatriation.

Further details may be obtained from the Director, Dr. T. E. Lowe.

Applications close with the undersigned on April 30, 1974.

G. B. CANHAM,
Secretary.

Baker Medical Research Institute,
Commercial Road,
Prahran, Victoria 3181,
Australia.

(193)

TECHNICIAN (Grade 3) required to assist in the running of a small research laboratory (4 workers) and to help with experiments on isolated tissues. Some knowledge of biology and chemistry essential. Salary in the range £1,650 to £1,920 plus £175 London Weighting.—Application form from Personnel Officer (Technical Staff FE2), University College London, Gower Street, London WC1E 6BT. (231)

MOLECULAR BIOLOGY AND BIOCHEMISTRY

UNIVERSITY OF CALIFORNIA, IRVINE

Applications and nominations are invited for permanent faculty positions available in September 1974. (1) A senior position in molecular genetics, (2) A junior position in an area of molecular biology, biochemistry or biophysics. Selection will be made on the basis of evidence of excellence in research and teaching. More consideration will be given to the candidate's qualifications than to the area of research. Send curriculum vitae, bibliography and list of three references to Dr Robert C. Warner, Chairman, Department of Molecular Biology and Biochemistry, University of California, Irvine, Irvine, California, 92664.

The University of California is an equal opportunity employer and encourages applications from women and minority group candidates. (204)

ROTHAMSTED EXPERIMENTAL STATION

HARPENDEN, HERTS. AL5 2JQ

BOTANIST/PLANT PATHOLOGIST to assist with work on infection of cereals by powdery mildew and testing for fungicide tolerance. Temporary post for two years. Minimum qualification: pass degree, HNC or equivalent.

Appointment in grade of Scientific Officer. Starting salary £1,318-£1,590 (depending on qualifications and experience) on scale to £2,177.

Applications, giving names of two referees and quoting Ref. 212, to the Secretary by February 8, 1974. (209)

UNIVERSITY OF YORK

DEPARTMENT OF BIOLOGY

LECTURESHIP IN ANIMAL BEHAVIOUR

Applications are invited for the post of Lecturer in Animal Behaviour from October 1, 1974.

Salary scale: £1,929-£4,548, with FSSU.

Six copies of applications (one from overseas candidates), naming three referees, should be sent by February 15, 1974, to the Registrar, University of York, Heslington, York YO1 5DD, from whom further details may be obtained. Please quote reference number 11/3092. (217)

UNIVERSITÄT ZU KÖLN GERMANY

INSTITUT FÜR GENETIK

E. COLI GENETICIST

A post-doctoral research position for work in association with Professor B. Müller-Hill will be available immediately. The appointment will be for two to three years. The salary will be on the scale of ca. 27,000,— DM per annum.

Applications, with curriculum vitae and the names of two referees, should be sent to Professor B. Müller-Hill, Institut für Genetik der Universität zu Köln, Köln 41, Weyertal 121, West Germany. (25)

THE UNIVERSITY OF ADELAIDE

Applications are invited for the following appointment:

TEACHING FELLOW IN ELECTRICAL ENGINEERING with interests in communication systems, signal processing, speech analysis, system assessment, power systems control, microwave engineering. Interaction with industry is encouraged. (1.2.74).

Salary scale: Teaching Fellow $A7,270 by $A286 (3) by $A285 (2) to $A8,698; a non-superannuated and non-tenured appointment.

Further particulars about this post and the conditions of appointment and other information sought will be supplied on request to the Registrar of the University, or to the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications should be sent in duplicate and giving the information listed in the Statement that will be supplied, to the Registrar, the University of Adelaide, North Terrace, Adelaide, South Australia, 5001. (223)

CELLULAR AND MOLECULAR BIOLOGY

A senior position at the rank of Full or Associate Professor is available in the Department of Biology, York University, Toronto, tenable July 1, 1974. Preferred areas of research interest are: genetics and biochemistry of development, cellular and molecular genetics, neurobiology. Exceptional junior candidates may also be considered. Salary negotiable and commensurate with rank.

Send curriculum vitae, reprints and names of three referees to Professor J. D. Friesen, Department of Biology, York University, 4700 Keele Street, Downsview, Ontario M3J 1P3, Canada. (114)

CSIRO

AUSTRALIA

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

RESEARCH SCIENTIST

DIVISION OF TEXTILE INDUSTRY

Geelong, Vic.

FIELD: **POLYMER CHEMISTRY**

GENERAL: The Organization's Division of Textile Industry located at Geelong, Victoria, is carrying out research concerned broadly with physical and chemical investigations related to wool textile processing. Facilities include well equipped laboratories for basic chemical and physical studies and full scale mill equipment for application of results.

DUTIES: To carry out basic studies on the application of polymers to wool and to work on the development of polymers for shrink-proofing and permanent press processes.

QUALIFICATIONS: A Ph.D. degree or equivalent qualifications together with demonstrable research ability. Experience in preparative organic chemistry, particularly polymer synthesis is desirable.

SALARY: Appointment will be made within the salary ranges of Research Scientist, or Senior Research Scientist, $A9,380–$A14,290 p.a.

TENURE: This is a three year appointment.

Applications stating full personal and professional details, the names of at least two professional referees and quoting Reference Number 464/558 should reach:

The Personnel Officer,
Australian Scientific Liaison Office,
64–78 Kingsway,
London,
WC2B 6BD

by the 15th February, 1974

Applications in the U.S.A. and Canada should be sent to:
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
Washington, D.C. 20036, U.S.A.

(228)

THE HUNTSMAN MARINE LABORATORY

SALMON RESEARCH CENTER

requires

FISHERIES BIOLOGIST IN CHARGE OF SALMONID CULTURE AND TRAINING

General—The newly formed Center is initiating a programme of research in Atlantic Salmon Restoration, with particular emphasis on genetics and selective breeding. There is an immediate opening for a key individual who will assume responsibility for the culture and culture training programme.

Duties—The successful applicant will assist in the design and construction of North America's first full scale salmon research-culture facility, will be responsible for implementing complex research programmes and advanced culture techniques, and will manage the Center's proposed advanced training programme for fish culturists.

Qualifications—Candidates should have a professional fisheries background and extensive experience in salmonid fish culture, in particular with Atlantic Salmon.

Salary—Negotiable.

Send résumé to: Dr John R. Calaprice, Director, North American Salmon Research Center, Huntsman Marine Laboratory, St Andrews, N.B., Canada. (205)

LA TROBE UNIVERSITY

MELBOURNE, AUSTRALIA

CHAIR OF MICROBIOLOGY

Applications are invited for the Chair of Microbiology. This is a Foundation Chair, and the Professor will initially be Chairman of the Department of Microbiology which the University intends to establish within the School of Biological Sciences in 1974.

Microbiology will be offered at undergraduate levels in the School of Biological Sciences in courses for the B.Sc. degree. It is planned that teaching will commence in March 1975. The appointee will be expected to participate in undergraduate and graduate teaching in general microbiology and to carry out research in his chosen field and be responsible for the administration and planning in the department. It is envisaged that teaching should begin at the second-year level. At present the school has departments of biochemistry, botany, genetics and human variation, and zoology.

The date of commencement of the appointment will be by arrangement. Applications close March 15, 1974.

The salary will be $A19,102 and membership of the University's superannuation scheme is required.

Further information and application forms are available from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, or from the Registrar, La Trobe University, Bundoora, Victoria, Australia 3083. (184)

GLASGOW
COLLEGE OF
TECHNOLOGY

Department of Ophthalmic Optics

Senior Lecturer and Lecturer in Ophthalmic Optics

Applicants should hold degrees or professional qualifications in Ophthalmic Optics. In the case of the Senior Lectureship, a Higher Degree or Higher Diploma together with appropriate experience will be required.

A special interest in clinical aspects, visual optics, ocular pathology or ocular anatomy will be an advantage. Those appointed will be expected to contribute to the development of, and teaching in, areas of ophthalmic study up to Honours degree and post-graduate level.

Members of staff are encouraged to pursue research interests and to read for Higher Degrees.

Salary Scales: Senior Lecturer—Up to £4,023 per annum, depending upon experience, plus £117 per annum for approved teaching training. Lecturer—Up to £3,201 per annum, depending upon experience, plus £117 per annum for approved teaching training.

Application forms and further particulars may be obtained from The Director, Glasgow College of Technology, North Hanover Place, Glasgow G4 0BA, and should be returned as soon as possible. (149)

MEETING

NATO—ADVANCED STUDY INSTITUTE

Exploitation of Seismological Network

A meeting organised jointly by the Royal Norwegian Council for Scientific and Industrial Research and Cranfield Institute of Technology, England, and sponsored by the NATO Science Committee, will be held from April 22nd to May 3rd, 1974, in Oslo, Norway.

The meeting will be concerned with the computational and mathematical procedures required for seismic array processing and the function of communication network used for this purpose.

It is intended to bring together those working in the fields of computation, communication, seismology and related areas—particularly those concerned with the use and implementation of seismic systems for earthquake and explosion identification.

Lecturers include: Dr. J. Bendat (U.S.A.); Prof. O. Dahlman (Sweden); Dr. E. Husebye (Norway); Dr. B. Kennett (U.K.); Prof. P. Kirstein (U.K.); Dr. R. Lacoff (U.S.A.); Prof. S. Mueller (Switzerland); Dr. D. Simpson (U.S.A.); Dr. H. Thiraway (U.K.); Dr. D. Weichert (Canada); Dr. P. Willmore (U.K.)

For further information and application for Fellowship to attend, please write to: Miss M. E. Williamson (Scientific Secretary), Computing Centre, Cranfield Institute of Technology, Cranfield, Bedford MK43 0AL, England.

ROTHAMSTED EXPERIMENTAL STATION
HARPENDEN, HERTS AL5 2JQ

PLANT PROTOPLAST AND TISSUE CULTURE STUDIES

A RESEARCH SCIENTIST is required to join a team in the Biochemistry Department working on the biosynthesis of amino acids in plant systems. The work will involve the selection of mutant plant lines having altered regulatory feedback characteristics. It is essential that the person appointed should have previous experience in higher plant protoplast and/or tissue culture studies. Minimum qualifications: first or upper second class honours degree.

Appointment in grade of Scientific Officer (£1,590 to £2,177) or Higher Scientific Officer (£2,076 to £2,667) depending on qualifications and experience. At least two years' appropriate experience is required for appointment in the higher grade. F.S.S.U. with a non-pensionable salary supplement of 4½ per cent to offset contributions.

Applications, naming two referees and quoting reference 217 to the Secretary by February 15, 1974. (244)

SCARBOROUGH, BRIDLINGTON, MALTON & WHITBY GROUP HOSPITALS

Applications are invited for the post of BIOCHEMIST (Probationary or Basic Grade) in the Group Pathology Laboratory, Scarborough Hospital, Yorkshire. Applicants should be about to graduate in Biochemistry, Chemistry, or one of the Biological Sciences, or hold the Associateship or Graduate Membership of the Royal Institute of Chemistry, or have relevant hospital experience. Excellent opportunities exist for in-service study leading to a higher qualification in particular the Mastership in Clinical Biochemistry. Whitley Council conditions of service apply.

Candidates are invited to visit this new laboratory by appointment with the Principal Biochemist. Tel. No. Scarborough 2121, Ext. 266.

Applications, together with the names of two referees, should be sent to:

The Consultant Pathologist,
Scarborough Hospital,
Scalby Road,
Scarborough, Yorks. (237)

BIOCHEMIST

required to work in department of Chemical Pathology on active endocrinological programme. Experience in radioimmunoassay an advantage but not essential. Opportunity to work for higher degree and for tr ing and experience in general clinical biochemis Salary according to Whitley scales.

Apply in writing to Prof J. R. Hobbs, Dept. of Chemical Pathology, Westminster Medical School, 17 Page Street, London, S.W.1. (238)

ROTHAMSTED EXPERIMENTAL STATION
HARPENDEN, HERTS AL5 2JQ

BOTANIST or BIOCHEMIST to investigate the function of growth substances in the development of sugar beet. Minimum qualifications 1st or upper 2nd class honours degree and some appropriate research experience (at least two years for appointment as HSO).

Appointment for two years in the first instance, in grade of Scientific Officer (£1,590 to £2,177) or Higher Scientific Officer (£2,076 to £2,667). F.S.S.U. Reference 210.

CHEMIST to assist with research on the effects of aerial pollution on crop plants. Minimum qualifications pass degree, HNC or equivalent.

Appointment for one year in the first instance in grade of Scientific Officer. Starting salary £1,318 to £1,590 (depending on qualifications and experience) on scale to £2,177. Reference 211.

Applications, giving full personal details and names of two referees to the Secretary by February 8, 1974. Please quote reference. (242)

ROTHAMSTED EXPERIMENTAL STATION
HARPENDEN, HERTS AL5 2JQ

BIOCHEMIST to join a team working on the enzymology and control of the biosynthesis of nutritionally essential amino acids in higher plants. Applicants should have a first or upper second class honours degree in biochemistry or a closely related subject.

Appointment in grade of Scientific Officer (£1,590 to £2,177) or Higher Scientific Officer (£2,076 to £2,667). At least two years' appropriate research experience is required for appointment in the HSO grade, but applications in the lower grade will be considered from people graduating in 1974. F.S.S.U. with a non-pensionable salary supplement of 4½ per cent to offset contributions.

Applications, naming two referees and quoting reference 216 to the Secretary by February 8, 1974. (243)

SUB-EDITORS

BUTTERWORTHS have vacancies for editorial staff in their Medical and Scientific departments. Previous experience of sub-editing manuscripts and all aspects of book publishing is essential. A wide scientific knowledge is necessary for applicants who wish to work on scientific books. The offices are situated in pleasant country surroundings at Borough Green, near Sevenoaks, Kent. The minimum salary for a qualified sub-editor is £2,405 per annum.

Please write, giving details of age, experience and qualifications, to Denise Lulham, Butterworth & Co. (Publishers) Ltd., 88 Kingsway, London WC2B 6AB. (241)

UNIVERSITY OF TORONTO

DEPARTMENT OF ZOOLOGY

ASSISTANT PROFESSOR (FOUR) OF ZOOLOGY

Applications are invited for four positions as Assistant Professor in the Department of Zoology, tenable from July 1, 1974.

The vacancies are in the fields of: 1. Genetics; Animal Behaviour; 3. Aquatic Invertebrates; 4. Vertebrate Physiology.

The successful applicants will be required to teach at both the graduate and undergraduate levels and to develop a research programme in their area of specialty.

The positions are established, with a minimum salary of $12,300 per annum, negotiable.

Applicants, who should have completed their Ph.D. and have Postdoctoral or similar experience, should send a curriculum vitae to Professor D. A. Chant, Chairman, Department of Zoology, University of Toronto, Canada M5S 1A1, to reach him not later than March 31, 1974. (236)

THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY

RESEARCH FELLOW/SPECIAL RESEARCH ASSISTANT

Applications are invited for the above vacancy to work in a research group investigating problems connected with condensation of vapours of immiscible liquids.

This forms part of a research programme sponsored by the Science Research Council to investigate the mechanism of condensation, to develop design procedures and improvements to equipment. A pilot plant test rig will be available. In the latter part the work investigations on production scale plant may be undertaken.

Applicants should have suitable qualifications in Chemical Engineering or Mechanical Engineering.

Salary within the range £2,058 to £2,373 per annum plus F.S.S.U. contributions.

Enquiries and letters of application should be addressed to:

Dr G. A. Davies,
Department of Chemical Engineering,
U.M.I.S.T.,
Sackville Street,
Manchester M60 1QD. (239)

RESEARCH ASSISTANT REQUIRED BY INSTITUTE OF CANCER RESEARCH, Belmont, Sutton, Surrey, to join team studying immunotherapy of human malignant disease. The candidate will be required to measure by *in vitro* techniques the immunological response of patients to malignant cells. Experience in tissue culture essential and experience in immunological techniques desirable. Salary according to experience, in scale £1,770 to £2,418.—Please apply in duplicate to the Secretary, Institute of Cancer Research, 34 Sumner Place, London S.W.7, giving the names and addresses of two referees, and quoting ref. no. 301/B/496. (190)

FELLOWSHIPS AND STUDENTSHIPS

THE ROYAL SOCIETY

SCIENTIFIC INFORMATION RESEARCH FELLOWSHIP

Applications are invited for a SCIENTIFIC INFORMATION RESEARCH FELLOWSHIP for research likely to improve scientific information services. The appointment is tenable for two years in the first instance from October 1, 1974, and may be renewable annually up to a maximum of five years. The stipend will be in the range £3,597 to £4,257 per annum, with superannuation benefits.

Forms of application are available from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG and applications are receivable by February 28, 1974. (212)

CSIRO

AUSTRALIA

POSTDOCTORAL FELLOWSHIPS (THREE)

DIVISION OF HORTICULTURAL RESEARCH

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELDS: **PLANT PHYSIOLOGY AND BIOCHEMISTRY**

GENERAL: The Division is concerned primarily with research on perennial horticultural plants of the temperate and sub-tropical zones, especially grapevines and fruit trees. The current program includes studies in: plant breeding, plant protection, canopy manipulation, drying of grapes, the storage of pome fruits, photosynthesis and fruit development.

The Division has laboratories at Adelaide, South Australia; Merbein, Victoria; and Hobart, Tasmania. The successful appointees will be required to take up duties in Adelaide, South Australia.

DUTIES: To collaborate in research projects concerned with the formation and early stages of development of fruits and nuts. Fields of interest include pollination, parthenocarpy, embryo formation, ovary wall enlargement in fruits and kernel development in nuts. Approaches to include light and electron microscopy, measurements of nucleic acids and proteins, assays of the activity changes in enzymes concerned with carbohydrate and lipid metabolism.

QUALIFICATIONS: Ph.D. degree in biology, botany, biochemistry or plant physiology or equivalent qualifications with specialized training in enzymology or cytology, together with demonstrable research ability.

SALARY: The appointments will be made within the salary ranges of Research Scientist or Senior Research Scientist, $A9,380–$A14,290 p.a.

TENURE: These Fellowships will be available for a period of two years.

Applications stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number 490/239 should reach:

The Personnel Officer,
Australian Scientific Liaison Office,
64–78 Kingsway,
London,
WC2B 6BD
by the 15th February, 1974

Applications in U.S.A. and Canada should be sent to
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
Washington, D.C. 20036, U.S.A.

(226)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF ELECTRICAL ENGINEERING

RESEARCH FELLOWSHIP

Applications are invited for a research fellowship financed by Arthur Holden & Sons Ltd., to work on the fundamental processes of surface coatings and electrostatic charging of powders. The successful applicant will join an established research group working on electrostatic deposition of materials with particular reference to industrial applications.

The fellowship is open to electrical engineers, physicists and chemists. A post-doctoral worker would be preferred but consideration would also be given to honours graduates having appropriate research experience. The post might be of interest to graduates with experience in surface physics.

The period of the appointment is two years and the salary will be up to £2,233 per annum.

Applications, giving details of age, qualifications and experience and the names and addresses of two referees, should be sent to the Deputy Secretary's Section (Ext. 2400), The University, Southampton SO9 5NH not later than February 28, 1974. Please quote reference No. Na 136/R. (220)

UNIVERSITY OF READING

UNIVERSITY RESEARCH FELLOWSHIPS

Applications are invited for one University Research Fellowship for independent research, normally of the value of £2,052 per annum.

Further particulars may be obtained from the Senior Assistant Registrar, Room 221, Whiteknights House, University of Reading, Whiteknights, Reading RG6 2AH, to whom applications (4 copies) should be sent by **February 11, 1974.** (179)

UNIVERSITY OF BRISTOL

LONG ASHTON RESEARCH STATION

AGRICULTURAL RESEARCH COUNCIL STUDENTSHIP

A studentship will be offered in 1974 for research in one of several fields relating to the work of the Station. For further details apply to the Secretary, University of Bristol, Research Station, Long Ashton, Bristol BS18 9AF. (202)

THE CITY UNIVERSITY

Department of Chemistry

Applications are invited for a

RESEARCH STUDENTSHIP

which is available for studies of the spontaneous ignition of hydrocarbon-air mixtures with special reference to the application of computer techniques. The value of the award will be at least equivalent to that of an S.R.C. Research Studentship and the successful candidate will be able to register for a Ph.D. degree.

Further details may be obtained from Professor C. F. Cullis, Department of Chemistry, The City University, St. John Street, London EC1V 4PB, to whom applications, together with the names of two referees, should be sent as soon as possible and in any case not later than February 5, 1974. (188)

UNIVERSITY OF HULL

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL FELLOWSHIP

Applications are invited for a Postdoctoral Fellowship (SRC) to work in collaboration with Dr R. Aveyard on the effects of electrolytes on aqueous solutions of surface active non-electrolytes. Research experience in thermodynamics, physical chemistry of electrolyte solutions, or colloid and surface chemistry would be an advantage.

The appointment, which is for two years, is immediately available at a salary of £2,037-£2,151 per annum.

Applications, giving details of qualifications, research experience, and the names of two referees, should be sent before January 31 to the Registrar of the University, from whom further particulars may be obtained. (218)

UNIVERSITY OF READING

UNIVERSITY POSTGRADUATE STUDENTSHIPS

Applications are invited for Postgraduate Studentships from persons who hold a first degree or who will have completed a first degree course by July 1974. The Studentships are tenable for a maximum period of three years from October 1, 1974, subject to annual renewal, and will be of the value of £695 per annum, together with payment of the Composition Fee and the fee for Associate Membership of the Students' Union.

Further particulars and application forms may be obtained from the Senior Assistant Registrar, Room 222, Whiteknights House, University of Reading, Whiteknights, Reading RG6 2AH, by whom applications (4 copies) must be received not later than **March 1, 1974.** (180)

UNIVERSITY OF OXFORD

GUINNESS RESEARCH FELLOWSHIPS IN MICROBIOLOGY

Candidates must be citizens of the United Kingdom, or the Republic of Eire or a Commonwealth country. They should have experience in research and be under the age of 28 at the time of appointment. Fellows are appointed for one year in the first instance at an initial stipend of £1,821 per annum. The appointment, which is tenable in the Microbiology Unit, Oxford, may be extended for a further period of two years and there is provision for F.S.S.U.

Applications including relevant details and the names of two referees should be sent before April 1, 1974, to Professor J. Mandelstam, Microbiology Unit, Department of Biochemistry, South Parks Road, Oxford OX1 3QU. (240)

UNIVERSITY OF CAMBRIDGE

BROODBANK FELLOWSHIPS

Election will be made in May 1974 to these Fellowships for the furtherance of research in Biochemistry or Biophysics with special reference to the Principles and Practice of Food Preservation. Tenure from a date and for duration to be determined but not exceeding total of three years. Fellow required to devote himself to full-time research in an approved subject normally at Cambridge. Candidature open, but preference to post-doctoral applicants. Fellow must become member of the University. Pensionable salary in the range £1,500 to £2,200 determined according to experience and qualifications. Compulsory superannuation. Grants may be given for research expenses. Allowed to give limited amount of paid teaching without reduction of salary.

Full details available from Secretary to the Managers of the Broodbank Fund, Department of Applied Biology, Downing Street, Cambridge CB2 3DX.

Candidates are advised to ascertain that there is a laboratory in Cambridge willing to accommodate research proposed.

Applications (ten copies) to reach the Registrary, University Registry, Old Schools, Cambridge, not later than March 1, 1974. Must outline research proposed and indicate bearing on principles and practice of food preservation. Must include full curriculum vitae, copies of papers published or references thereto and names and addresses of at least two referees. (192)

AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

DEPARTMENT OF DEVELOPMENTAL BIOLOGY

Applications are invited for a Post-doctoral Fellowship and Research Scholarship to form part of a newly-constituted research group headed by Professor Brian Gunning.

POST-DOCTORAL FELLOWSHIP: Consideration will be given to suitably qualified applicants with experience combining electron microscopy with physiology or biochemistry, interested in ultrastructure of algal cells or of transport systems in higher plants, or applications of S.T.E.M. and energy-dispersive analysis of X-rays.

Post-doctoral Fellows are normally appointed for a minimum of one year and maximum of two. The salary range is $A8,698 to $A11,901 per annum and superannuation on the F.S.S.U. pattern is available. Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra.

The closing date is **February 11, 1974,** and applications should be sent direct to Dr. B. E. S. Gunning, Botany Department, Queen's University, Belfast 7BT 1NN, Great Britain. Applicants should ask three referees to write immediately to Dr Gunning.

RESEARCH SCHOLARSHIP: A Ph.D. scholarship is available for work on aspects of translocation in leaf tissues, involving investigations by conventional, quantitative, and analytical electron microscopy.

Research Scholarships are awarded for three years and provide a yearly tax free allowance of $A3,290 with extra allowances for dependent spouse and children. Return fares to Canberra and a grant for removal expenses are normally paid. Assistance with accommodation is available.

Application forms are available from the Acting Academic Registrar, P.O. Box 475, Canberra City, ACT, 2601, Australia, and should be returned completed by **February 8, 1974.** (196)

THE WELLCOME TRUST

TRAVELLING RESEARCH FELLOWSHIPS TO WESTERN AND EASTERN EUROPE 1974/75

Applications are invited from British research workers for the award of Wellcome-European Research Fellowships for 1974/75. These awards include the Trustees' special fellowships to Denmark, Germany, Hungary, Norway and Sweden.

The object of these fellowships is to encourage working visits to countries in Western and Eastern Europe by investigators in any branches of the natural and clinical sciences which have a bearing upon human or animal medicine. Candidates whose research is related to clinical problems will be given preference. These fellowships, which are normally for one year, are intended for graduates from the United Kingdom who already have some post-doctoral research experience. Stipends, according to age and experience, are within the range of £1,930 to £3,875 per annum. A special cost of living allowance applicable to the country in which the candidate wishes to work is given where necessary. Travelling and some incidental expenses are provided in addition.

The candidate must make his own arrangements with the department in which he proposes to work, and written evidence must be submitted confirming his arrangements. Application forms may be obtained from the Assistant Director, The Wellcome Trust, 52 Queen Anne Street, London, W1M 9LA. Completed application forms must be returned by March 31, 1974. (213)

CITY OF BIRMINGHAM POLYTECHNIC

THE BIRMINGHAM SCHOOL OF ARCHITECTURE

RESEARCH FELLOWSHIP

Applications are invited from scientists, architects and other suitably qualified persons, to undertake research in the Architectural Science Unit. The appointment is initially for one year but may be renewed annually for up to three years.

Salary scale: Lecturer II: £2,515-£3,243.

Full details and application forms (to be returned by February 1, 1974) from: The Staffing Officer, City of Birmingham Polytechnic, Room T.310, Perry Barr, Birmingham B42 2SU. (215)

POTATO MARKETING BOARD

POSTGRADUATE STUDENTSHIP AWARDS 1974

Applications are invited for the above studentship awards which are made to enable graduates holding first or upper second class honours degrees in pure or applied science to undertake further training so that they may conduct research at universities or research stations in this country on problems facing the potato industry. Applications are specially invited from graduates concerned with the production and marketing of the crop, the physiology and biochemistry of potatoes in relation to resistance to damage and improved quality, and the reduction of wastage caused by damage during the harvesting and handling of the crop.

The awards are tenable for up to three years at selected universities and research establishments in Great Britain and are valued at £800 per annum (tax free) plus educational fees.

Applications receivable by March 1, 1974, should be made on forms obtainable from the Secretary, Potato Marketing Board, 50 Hans Crescent, London SW1X 0NB. (178)

LECTURES AND COURSES

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF LANDSCAPE ARCHITECTURE

M.A. COURSE IN LANDSCAPE DESIGN

Applications are invited from graduates with good academic qualifications in disciplines related to landscape architecture. The course is of two years' duration and is recognised as giving exemption from Parts 1, 2 and 3 of examinations leading to professional membership of the Institute of Landscape Architects.

For details and application forms apply to the Faculty of Architectural Studies, The University, Sheffield S10 2TN. Please quote R 13/G. (182)

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BACK RUNS OF JOURNALS

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Cannon House, Park Farm Road,

Folkestone, Kent, England.

Tel.: Folkestone 57421. (1)

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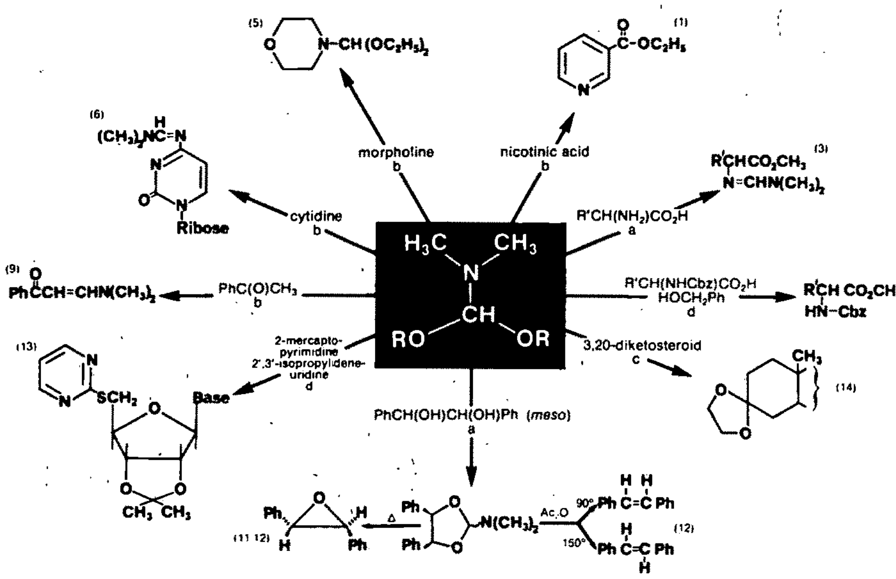
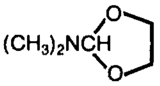


Reagents: a = $(CH_3)_2NCH(OCH_3)_2$; b = $(CH_3)_2NCH(OC_2H_5)_2$; c =  ; d = $(CH_3)_2NCH[OCH_2C(CH_3)_3]_2$

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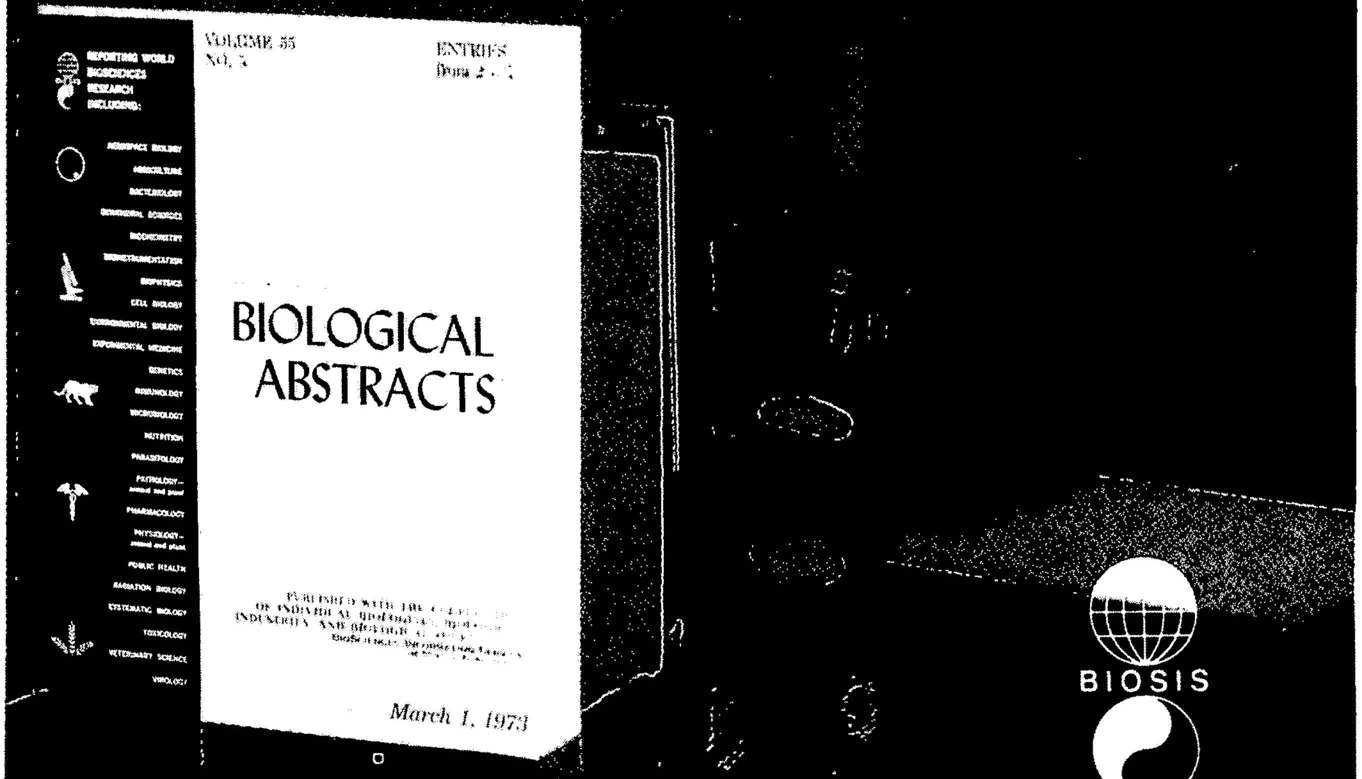

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A fuller guide appeared in *Nature* (**246**, 238; 1973).

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Seven questions for the energy secretary

Britain now has a Department of Energy whose Secretary of State is Lord Carrington, previously Secretary of State for Defence. The brief of this new department is, in the words of the Prime Minister, "to concentrate on the development of the coal industry on nuclear power and on our offshore oil and gas resources at home, as well as on those tasks of working together with other oil-consuming countries and with the oil-producing countries on the international aspects of the energy problem".

The following energy issues will ultimately deserve his or his successor's attention.

How permanent is the department to be? It is unlikely that the problem of finding adequate, reasonably priced energy sources is going to go away before the turn of the century. Many scientists and technologists could become enthused by the thought of a long term commitment to a strong government department pursuing such desirable objectives as rethinking energy strategy. They could not face, however, being pushed around from ministry to ministry each time the Cabinet was reshuffled.

Can energy be removed from the political arena? There are many aspects of energy policy which merit serious discussion but the subject can easily become a political football. The inferior quality of the recent Commons debate on the fuel shortage does not bode well for informed non-partisan discussion in the future. Lord Carrington is, of course, a political creature holding simultaneously the post of Chairman of the Conservative Party. At the moment he is probably as obsessed with the thoughts of election strategies as with energy policy and in the long run it is desirable, assuming he wishes to remain as Secretary of State for Energy, that he does not divide his talents, particularly with such a politically exposed position.

Can some order be brought into research funding? Some weeks ago we reported on an energy strategy devised for the United States (**246**, 370; 1973). An important feature of it was that it was not intended to be competitive. Funding for coal was not to be at the expense, say, of funding for validating the nuclear option, nor was the coal industry in the process of its research expected to denigrate the nuclear industry. A strong impression left by many British pronouncements is that developments are largely made for this very purpose. It is therefore highly desirable that an urgent new look at funding mechanisms be made, in order to impose, if necessary, a degree of overlordship by an energy department.

Consequent on this, why not a think tank? Britain is not short of experts in almost all individual fields of energy research and use. It is, however, desperately lacking in people with wider vision. The reasons need not be repeated here. Yet without such people, and they should not necessarily be civil servants, an unreasonable load is placed on a minister in making decisions. A think tank would at least be a means of educating a wider range of people in strategic thinking.

Cannot conservation efforts be improved? Remarkably little has been done in the way of education for saving fuel and yet there are very attractive opportunities. Why does Lord Carrington not visit a power station and invite, in front of television cameras, all viewers to turn off something electrical? The drama of a meter needle moving under the influence of one's own action in the home would rival Mr Geller's activities.

How will Britain react to the Kissinger energy initiative? At the same time that it was being 'warmly welcomed', Britain and France were doing bilateral deals wherever possible—deals which, however desirable in the short term, cannot have a long term future within the spirit of international cooperation. However cynical one may be about the United States leading such an initiative while consuming energy so greedily and often wastefully, in the long run someone had to recognise that energy is so much a supranational and supra-oil company affair that it has to be dealt with internationally.

And, this being so, should Britain take steps to safeguard the interests of other countries, particularly developing ones, for whom increased energy prices are much more economically damaging than for industrialised countries? The early formulation of an international attitude to energy is possibly the most urgent need there is today. It will be of great interest to see whether the big energy consumers can see beyond their own industrial landscape.

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From *Nature*, **9**, 237, January 29, 1874.

Is population a ministerial responsibility?

Mr D. R. Cope of the Institute of Planning Studies, University of Nottingham, questions the need in Britain for a minister with special responsibility for population matters.

THE announcement, by the Prime Minister, Mr Edward Heath, just before Christmas that Mr James Prior, Lord President of the Council, is to be minister with special responsibility for population matters means that one of the major recommendations of the Population Panel, set up in November 1971, has become government policy (*Report of the Population Panel,* HMSO; 1973). Population is by no means Mr Prior's only responsibility, as several pressure groups campaigning for a separate ministry were quick to point out, but his appointment raises the question of what are the areas of action open to a minister for population and what should be his priorities.

As the panel noted, quite a wide range of government activities, especially health, social welfare, fiscal and planning policies, may have an impact on population trends, but to date no government has formed policies with particular regard to the possible impacts. Is this situation likely to change?

The current demographic position in Britain is that no serious economic or social problems which could be directly attributed to either its population size or growth rate are likely to emerge in the next thirty or forty years. Present fertility behaviour will, of course, have an effect on population levels in the first decades of the next century and a minister of population will be concerned with what are, for economists and social planners, very long term futures. For politicians, such a long term concern is almost unprecedented and the potential unpalatability of a current policy designed to affect the demographic situation in the next century would be so great as to deter any government from incorporating an overt population policy in its programme of action.

Will the British government pronounce an opinion on an 'optimum' national population? The considered opinion of most academic demographers, such as Professor D. V. Glass of the London School of Economics, is to dismiss the concept as providing no useful base for policy making. Different criteria produce a congery of optima, many of which conflict with each other. Like the legendary castle which always stayed on the horizon no matter how far or fast travellers rode towards it, an optimum population, if it could be defined, would be unobtainable. In the time taken to achieve a stated population, external circumstances would almost certainly have altered to give rise to another optimum.

The major field of potential action in population matters is in family planning policy. The present government has already incorporated an extended service in the health service reorganisation bill, though it was motivated as much by considerations of general social welfare as by any demographic consequences. It is well known that even if every birth in Britain were to be 'wanted'—if there were to be perfect use of contraceptive facilities—current family size patterns mean that the population would continue to grow. Demographically, a policy of freely available contraceptive facilities and a free abortion service to take care of cases of contraceptive failure or unavailability would have the greatest potential impact. Abortion can be a very effective agent in influencing population patterns but the sensitivity of the subject is again likely to deter governments from too firm or blatant a policy relying on it.

There are really only two areas in which a population minister could identify immediate action needs: in demographic research and in publicity and information. Despite recent increases there is still far too little serious demographic research being carried out in Britain. Over the past few years many unsubstantiated assertions have been made on the relationships between population and the economy and environment, and about the impact of human numbers and densities on social and psychological well being. The need now is for research to test some of these interrelationships, especially factors affecting fertility behaviour. Demographers are also hopeful that Mr Prior's appointment will make it difficult for the government to cancel the proposed 1976 census on the grounds of economy, as a ten year intercensal gap is manifestly inadequate for determining changes in trends.

The Population Panel placed great emphasis on the need for government action on publicity and information about population trends and patterns. Obviously, a family planning service will be fully successful only if all those who could use it are aware of it and do use it. The panel, however, looked beyond such simple publicity and argued the necessity of bringing home to the public the need to consider the aggregate consequences of their family building behaviour, despite the possible conflict of these consequences with individuals' preferences. This reminds one of government attempts to do much the same in the context of income restraint and no doubt the public response would be very similar.

James Prior, Lord President of the Council

It will be interesting to see how long the position of minister with responsibility for population matters survives, given the very limited possibilities associated with it. Demographic trends may even rapidly make it superfluous if the position is seen as being specifically concerned with problems of population growth. As yet the post-1964 downturn in the numbers of live births shows no signs of reversing and despite allusions to the 'New York blackout syndrome' (which was a myth anyway, according to Udry, *Demography,* **7**; 1970), current economic difficulties may well lead to an even greater decline in birth numbers with couples avoiding additional expenses by postponing births. Widespread advance warnings of economic rigours and the much greater use of the oral contraceptive pill mean that Mr Prior will not have to concern himself with a marked upturn in births in the last quarter of this year.

international news

Credibility gap worries US energy office

Colin Norman, Washington

After being deluged with dire warnings about the energy crisis that was supposed to hit the United States this winter, many Americans are now beginning to wonder whether there is a crisis at all, or whether the whole thing is an elaborate plot by the oil industry to drive up prices and profits. So far, there have generally been adequate supplies of gasoline and heating oil, cities are still brilliantly lit at night by hideous advertising signs, and life is proceeding more or less normally for most people. About the most tangible sign of energy shortages is that gasoline and heating oil have increased in price.

According to William Simon, Director of the Federal Energy Office, his biggest problem in the next few months is the growing public scepticism about the energy crisis. He is anxious because such scepticism could lead to backtracking on conservation measures which have so far turned out to be remarkably effective in curbing demand, and that in turn could precipitate a full-blown crisis before the summer arrives. And the problem of maintaining public support will become even more difficult once Arab oil again starts flowing into the United States. In fact, the Administration is so concerned about the matter that President Nixon made a radio address last weekend during which he warned that "if we permit ourselves to slack our efforts, to slide back to the wasteful consumption of energy, then the full force of the crisis will be brought home to Americans in a most devastating fashion, and there will be no longer any question in anyone's mind about the reality of the crisis".

The scepticism stems not only from the fact that there has been little discomfort so far in the United States, but also from a series of reports of record profits in the oil industry, rumours of tankers sitting outside ports waiting for oil prices to creep up before they unload their cargoes, and allegations that storage tanks at refineries are brimming with heating oil. But perhaps the most damaging blow to the credibility of the crisis is the growing public awareness of the fact that the government has been forced to rely almost exclusively on the oil industry itself for statistics about production and stocks of oil. As Mr Silvio O. Conte, a Republican Congressman from Massachusetts, put it last week, that is like "putting a fox in to watch the chicken coop".

The Administration is thus beginning a campaign to assure the American public that the crisis is real, and that conservation measures are still necessary. First, President Nixon promised in his radio message that he will send a bill to Congress which would impose a windfall profits tax on oil companies to force them either to turn over excess profits to the government or to invest them in exploration for new sources of energy. And second, the Administration is sponsoring a bill which will, in Nixon's words, "require the oil companies to provide a full and constant accounting of their inventories, their production, their costs and their reserves". And, in the meantime, Simon has ordered a full audit to be conducted on the companies' figures. Those measures should, at least, help dispel some of the public distrust over the figures.

But, although the government has been relying on industry data for its analysis of the energy situation, there is still good reason to believe that the shortages are real, and are not solely the product of an inspired public relations exercise by the oil companies. As S. David Freeman, Director of the Ford Foundation Energy Policy Project, suggested last week, the companies have certainly been making a lot of money out of the situation, and the data they have supplied through the American Petroleum Institute may not tell the whole truth and nothing but the truth, but he suggests that they still indicate the need for gasoline rationing to be imposed in the spring.

And there is also good reason to believe the Administration's assertions that if the American public slackens in its conservation efforts, a severe crisis could still develop. For one thing, a variety of data supplied by the electricity industry, by the oil companies and by independent analyses all attest to the fact that conservation efforts have been remarkably successful in decreasing demand, even if the unusually warm weather in the last quarter of 1973 is taken into account. And for another, a lot of oil has been flowing into the United States through the embargo, and this is now beginning to dry up—and even if the embargo is suddenly lifted, it will take several weeks for the oil to reach United States ports.

One of the most direct indicators of demand for energy is the amount of electricity supplied to consumers. According to data produced by the Edison Electric Institute, at the end of last year, consumption of electricity throughout the United States was averaging about 1.5% below that of a year previously. Moreover, during the week ending on January 12, demand dropped by 4.1%. Compared with an expected increase of about 7%, those figures show a very substantial decrease in demand.

In his radio address last weekend, President Nixon also cited a sheaf of statistics to show that demand for other forms of energy has declined equally dramatically. Consumption of gasoline in December was reduced by 9% below expected levels, for example, natural gas consumption was down by 6% compared with last year, and he also referred to a study in the North East which showed that some 19,000 households had reduced their heating oil consumption by an average of 16% compared with last year, even after adjustments had been made for the warmer weather.

The Administration still reckons however, that during the first three months of 1974, supplies of oil will fall short of previous demand by about 2.7 million barrels a day, or about 15%. This must be accounted for either by rationing or by conservation measures. Heating oil is already being allocated to parts of the country which are especially hard hit by the oil embargo (in other words, it is being rationed at the wholesale level), and last week the Federal Energy Office published details of a gasoline rationing plan which will be brought in if necessary, although no earlier than March.

In any case, President Nixon declared last weekend that he is opposed to gasoline rationing, and will avoid bringing it in if at all possible. He said, for example, "if this voluntary cooperation (in reducing consumption) continues, . . . we can avoid gas rationing this spring . . . and you can be sure that I will do everything in my power to achieve the goal of avoiding gas rationing."

Pollution research in a sorry state

Colin Norman, Washington

THE federal government's research and development programmes for cleaning up water pollution are starved of money, poorly managed and badly coordinated, according to a massive study carried out by the General Accounting Office (GAO). Unless the programmes receive more funds, the GAO said in a report made public last week, the goals set by the 1972 Water Pollution Control Act will not be met. Charges of financial undernourishment are commonplace in Washington, but in this instance they gain considerable credibility from the fact that the GAO, which is an investigative agency of Congress, has no axe to grind since it does not have a direct stake in the matter.

The Water Pollution Control Act, which was passed by Congress in December 1972, specifies that waterways in the United States should be clean enough to swim in by 1983, and that no pollutants at all should be discharged into navigable waters by 1985. Those are tough goals to meet, and they will clearly require massive investment of funds in sewage treatment plants. They will also require a vigorous programme of research and development on new control technologies.

Far from pursuing a vigorous programme, however, the Administration has cut some research budgets and failed to disseminate results from the studies which are being carried out. Moreover, the GAO charges that no overall strategy for research and development has been worked out by the Environmental Protection Agency, and the research is therefore not sufficiently geared towards meeting the goals specified in the Water Pollution Control Act.

Between July 1969 and June 1973, about $495 million was spent by twelve federal departments and agencies on water pollution research, some $238 million of which was spent by the Environmental Protection Agency (EPA). Although the EPA is supposed to be the focal point in the federal government for pollution control research and for enforcing federal environmental laws, its water pollution research budget was actually cut by the Administration in the 1973 fiscal year, from about $50 million to $42 million. Meanwhile, the water pollution research programmes of other agencies doubled between 1969 and 1972, increasing from $36 million to $71 million.

A prime objective of the EPA's research programme should be to minimise the costs of treating municipal sewage, because huge sums of money will be spent in the next few years on treatment plants—the 1972 Act, for example, authorises expenditures of $18,000 million between 1973 and 1975 for grants to help in the construction of sewage plants. A small percentage saving from research would clearly save large sums of money.

But the GAO report points out that although $3,000 million was earmarked for municipal sewage grants in 1973, only $9.5 million was earmarked for the development of control technologies. That translates to an investment of only 0.3% in research, and compares with a rate of 8% in the Department of Transportation's urban mass transportation programmes, and a rate of about 4% in industry, the GAO report notes.

A similar situation exists in another important EPA research programme—research aimed at determining how pollutants get into water, what happens to them, and what their effect is (so-called process and effects research). The GAO found that because of funding limitations and poor management, important research needed to establish water quality standards has been delayed, and research on thermal discharges has been inadequate. As far as the latter programme is concerned, the GAO report notes that thermal pollution is likely to be a rapidly growing problem as the number of power plants increases, but research is proceeding slowly, and according to the director of one EPA laboratory, the agency has not even begun to solve the thermal pollution problems likely to be present in 20 years' time.

The GAO also found that there is a lack of coordination between the government's research and that conducted by industry, to such an extent that "we were informed (by representatives of industry) that industry was reluctant to reveal to EPA the level of technology developed to control pollution because (EPA) might speed up its enforcement action and industry might suffer a financial loss".

What needs to be done to alter this sorry state of affairs? Apart from increasing expenditures on research and development, the GAO report recommends that EPA should draw up a research and development strategy which would estimate the amount of money needed to meet the goals set out in the Water Pollution Control Act. The Office of Management and Budget should designate a federal agency as the focal point for coordinating research, and for disseminating research results, and ensure that EPA gets full cooperation from other agencies in drawing up its research and development strategy.

As for the Congress, the GAO suggests that since it is doubtful that waterways will be cleaned up by 1983 with present research funding levels, some thought should be given to increasing research appropriations. The

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Sherman quits NIH

Colin Norman, Washington

A TOP official at the National Institutes of Health has resigned in frustration over the Administration's policies for biomedical research in general and for NIH in particular. Dr John Sherman, Deputy Director of NIH since 1968, and an NIH employee since 1953, will be leaving early in March to join the staff of the Association of American Medical Colleges, a Washington lobbying organisation.

Sherman said in a telephone interview last week that he has resigned "because of a number of tangible and intangible things" which are eroding the prestige of NIH and "making it a less attractive place to do research in". Chief among Sherman's complaints are the fact that the Administration has cut the number of personnel positions at NIH, and because he finds a lack of understanding between officials in the agency and administrators in the Department of Health, Education and Welfare.

As far as staffing is concerned, Sherman pointed out that personnel have been cut back at a time when the budget has been expanding, and when greater emphasis is being placed on contract research as opposed to grants. Contracts take more time to administer, and therefore require more staff.

For some time, there has been general discontent at NIH over policies dictated by the Nixon Administration. Many of the complaints have revolved around budgetary problems, because the Administration cut many of the institutes' research budgets last year, and also because Congress and the Administration failed to agree on a budget for the agency for almost 18 months. But Sherman's complaints go deeper than simple lack of money. "There is a sort of a sense of negativity (in the Department of Health, Education and Welfare), and a lack of understanding between us and the decision-makers", he said, and many of the frustrations stem from the strict management control that the Administration is trying to place on basic biomedical research.

Sherman first joined NIH in 1953, he has been an Associate Director of NIH for Extramural Programs, and was appointed Deputy Director in November, 1968. When Dr Robert Q. Marston resigned as NIH Director a year ago, Sherman was appointed acting director for some six months.

problem is, however, that the Administration needs to be persuaded to spend money for water pollution control which has been earmarked by Congress. So far, the Office of Management and Budget has released less than half of the money for sewage treatment grants which Congress authorised in the 1972 Water Pollution Control Act.

New cyclotron for neutron therapy

John Hall

Because of Hammersmith Hospital's success in the use of neutron therapy for the treatment of cancer a second British hospital is to be supplied with a compact cyclotron. The Medical Research Council (MRC), the Scottish Home Office and Health Department, and the Cancer Research Campaign will jointly finance a unit costing £819,000 at the Western General Hospital Edinburgh, which should be ready to treat patients by the summer of 1976. Hammersmith Hospital has been a world leader in the use of fast neutrons for cancer treatment and if clinical trials at both centres are as successful in the future as they have been to date, the Department of Health could well be persuaded to make a big investment in this type of treatment.

The first cyclotron in the world to be entirely devoted to medical research was installed at Hammersmith in 1952. After extensive research in the laboratory, clinical trials in the treatment of advanced cancers were started in 1969. Randomised trials conducted alongside conventional programmes of X-ray treatment showed promising results in the treatment of cancers situated in the head and neck, although patients often died from secondary cancers which had spread before the primary source could be destroyed.

Radiotherapy in this area works on the principle that malignant tissue is more sensitive than healthy tissue to destruction by X-rays and γ rays. Where there is a low oxygen concentration in the tissue, caused perhaps by the presence of the tumour, there is, however, a reversal of the effect and the cancer is often able to grow again after partial destruction. The effectiveness of neutron therapy depends on the ability of the neutrons to destroy cancer cells even in the absence of oxygen.

After closely following the trials at Hammersmith, workers in the United States decided a year ago to adapt three of their large cyclotrons used in physical research for cancer treatment and other centres will be established in France, Germany, the Netherlands and Japan.

The Edinburgh cyclotron will produce neutrons with twice the energy available at Hammersmith and will be made more versatile by the inclusion of a steering device which will allow a site to be attacked from several different positions.

The new installation will be an outstation of the MRC's Hammersmith Cyclotron Unit, which is directed by Mr Derek Vonberg. The clinical programme will be directed by Professor W. Duncan of the Department of Radiotherapy (University of Edinburgh) and the clinical trials (which at Hammersmith were led by Dr Mary Catterall) will be supervised by a special committee on neutron therapy of the MRC, on which the Cancer Research Campaign will be represented.

What is wasted?

Even when we can actually lay hands on fuel for motor systems and power generation we waste a vast amount of the energy potential contained in the raw materials. In his Inaugural Lecture at the University of Leeds, Professor Alan Williams examined Britain's power station wastage and compared the fuel efficiencies of various methods of transport.

He finds that the average power station supplying 2,000 MW of electricity also produces 4,000 MW in steam and warm water which is generally not put to any use, so that even during the energy crisis Britain can only benefit from 45% of all fuel actually burnt.

The average engine is still less than 20% efficient in converting fuel into useful motive power and a comparison of different transport methods in terms of passenger miles per gallon showed a fully loaded train to be ten times more efficient than a jet aircraft and fifteen times more efficient than Concorde.

Professor Williams, Livesy Professor of Fuel and Combustion Science, considers that the efficiency of liquid fuel engines and power stations is capable of being improved by a further 10%. Of the 25,377,000 tons of liquid fuel used for transport in 1972, something like 74% was used by road vehicles operating at low efficiencies. Although in theory a diesel engine might have a thermal efficiency of 37% and a petrol engine 25% (17% in the case of a Wankel), in fact only 15% to 20% of their fuel is converted into useful energy.

Bringing NASA down to Earth

Colin Norman, Washington

Buried in the pile of bills, resolutions and impeachment motions facing members of the US Congress who returned to Washington last week is a little-noticed item which could radically alter the National Aeronautics and Space Administration (NASA) and create yet another new focus for federal science policy-making. The bill, which was introduced into the Senate last September, is likely to be the subject of public hearings in February and the topic of considerable debate thereafter.

Called the "Technology Resources Survey and Applications Act" and numbered S2495, the bill would give NASA some $200 million a year to help apply science and technology to pressing domestic problems, partly by enlisting the help of the depressed aerospace industry. It would also establish a Cabinet-level committee to survey the United States' scientific resources, and to plan how those resources could best be used to improve such items as health care delivery, sewage treatment and other domestic services.

The bill would thus greatly extend NASA's range of activities in fields other than space research, and it would add considerable impetus to the much-discussed move toward spending federal research and development dollars on more socially useful projects. But it is also likely to run into opposition from the Administration, and its prospects are uncertain.

In many respects, the bill is similar to Senator Edward M. Kennedy's National Science Policy and Priorities Act, which was passed by the Senate in 1972 but which died from neglect in the House of Representatives. Kennedy's bill would, however, have placed responsibility for supervising and sponsoring such domestic research and development programmes in the National Science Foundation—at best an unlikely location, but one which would have placed the whole effort under the jurisdiction of Kennedy's NSF Subcommittee. NASA is clearly a more logical home for such an enterprise because it already has an office concerned with technology utilisation, but even so, some bureaucratic turf fighting would be inevitable if the bill were passed in its present form.

For one thing, the National Science Foundation is now supposed to have responsibility for surveying the government's efforts in science and technology and for advising on how federal money should be spent on research and development. It would be unlikely to yield a good part of that responsibility to the Cabinet-level committee proposed in the bill. And there is also the problem that the Administration would not take kindly to such a large spending commitment in such difficult financial times.

What are the chances that the bill will be passed? Staff members of the Senate Committee on Aeronautical and Space Sciences are hoping to get some hearings underway in February. But one problem is that the bill has also been referred to the Senate Commerce Committee whose chairman, Warren Magnuson, is the bill's chief sponsor. Unless some formula can be worked out for both committees to consider the legislation simultaneously, its passage through the mill will be slowed down. Moreover, even if the bill is passed by the Senate before Congress adjourns for the October Congressional elections, there is no sign of interest in the House of Representatives. The House Science and Astronautics Committee, for example, has its plate full with energy matters, and would be unlikely to take up the bill in time to get it passed by the House this year.

Nevertheless, the bill is likely to spark considerable discussion in the scientific community, just as Kennedy's bill did two years ago.

No astronomical ghosts laid

Edward Phillips

Dr David Evans, the Welsh-born optical astronomer, sees himself as the *enfant terrible* of British astronomy, although some would say *vieux terrible*. After many years in British and South African astronomy he now works at the University of Texas, but last week he was in London and at the monthly meeting of the Royal Astronomical Society. And for once the society forsook its normal academic fare to allow Dr Evans to present his views on the management of British optical astronomy.

Dr Evans seemed to be attributing what he called "the depressed state of British optical astronomy" to two factors. First, he blamed "overbureaucratisation" which he said resulted in the voice of optical astronomy emanating from administrators instead of from the scientists working at the observatories. And, second, he recognised "an unfortunate relationship between optical and theoretical astronomers", there being a tendency to disparage any work which originates too far from what he termed the Cambridge-Caltech axis.

All this was greatly enjoyed by the younger astronomers present, while their elders were notably less amused. But after this cracking start Dr Evans lapsed into a panegyric for astronomy at the University of Texas, in particular the McDonald observatory at Fort Davis which he put forward as the ideal site for the proposed Northern Hemisphere Observatory.

In the circumstances the president of the society, Professor D. E. Blackwell, was probably being benevolent in ruling that there was insufficient time for discussion, although Sir Bernard Lovell was allowed to say a few words in support of British optical astronomy.

Unfortunately, though, this meeting, which could have served as a much needed exorcism after all the troubles that have beset British optical astronomy, fell far short of achieving any useful purpose. The Royal Astronomical Society is, however, planning a meeting where officials of the Science Research Council will be able to explain the latest policies in astronomy. Maybe this will be the occasion for casting out a few devils.

Keeping a non-stop world

Having sacked the Earth to the point where (arguably) we are virtually bankrupt of fossil fuels, it is good to see a politician draw the line at actually stopping the planet from spinning in the pursuit of new energy sources.

In a House of Commons debate on tidal power British Member of Parliament Mr Tam Dalyell asked whether or not the government was prepared to talk about tidal energy at an international level since, he observed, there is a serious question of reduced Earth spin if several countries embarked on such schemes. Mr Dalyell also asked whether or not there would be adverse effects on the ocean bed and whether such fears need be harboured if there were a two-basin estuary scheme.

His point in raising these questions, apparently, was to demonstrate that people who proposed tidal energy schemes were aware of the attendant anxieties and, he submitted, it would be silly not to express a certain sensitivity towards them. Which of course is true, though, it must be added, it would not be ever so silly since the estimated time required for tidal harness schemes to stop the Earth's spin is something in the region of 10 million years.

correspondence

Energy

Sir—The United States is feeling the energy pinch and is beginning to realise that pertoleum, along with other critical materials, cannot last forever. This is causing us to intensify our efforts toward alternate energy sources and to examine how we can reduce consumption and increase reclamation. It is also causing critics, both locally and abroad, to accuse America of the 'mindless' utilisation of energy (*Nature*, **246**, 116; 1973). Statistics are quoted about our small population using a third of the world's energy and implying that this energy is mostly wasted or used for personal comfort and enjoyment.

I will concede that we have wasted resources, most recently in Vietnam, and that we drive too much, have too much throw-away packaging, and more air conditioning than we really need. But I argue that the majority of the energy has been used productively. America has exported energy to the four corners of the world in the form of wheat, rice, soy beans, cotton, lumber, machine tools, tractors, airplanes, locomotives, and medicine.

We have exported American know how, fed and clothed both allies and enemies after World Wars I and II, and rebuilt their factories. Their factories today are more modern than our own. For much of this we have received little or no payment—yet these activities have caused depletion of our soil, natural resources, and treasury. I wish our critics in England, France, Russia, Germany, Japan, China, and the Middle East would remember some of these things when preparing their editorials.

In some instances we may have been guilty of exploitation and of not minding our own business. But, when it comes to energy, perhaps we have not used more than our fair share, considering the billions of BTU's used to make or grow the products we have given away.

Yours faithfully,

E. A. Aas

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news and views

New view of deep earthquakes

Conventional wisdom has it that an earthquake is a sudden rupturing across a fault plane, the total duration of the event being roughly the maximum distance along which slippage occurs divided by the rupture velocity of several kilometres per second. It is also very widely believed that a 'mechanism' can be associated with every earthquake—a quadrantal distribution of rarefactions and compressions related to the orientation of the fault plane and the slip vector. A paper by Dziewonski and Gilbert in this issue of *Nature* (page 185) raises some fascinating questions about this simple and highly successful model of earthquakes. It seems that deep quakes can be preceded by over a minute of slow compression.

For the past ten years it has been possible to study radiation patterns of earthquakes by the use of long period (10 to 100 s) seismometer recordings of body waves. The signal begins sharply—it seems to have both a precise starting time and an unambiguous polarity. The observed distribution of these polarities over a notional sphere surrounding the quake is in almost all cases entirely concordant with a 'double-couple' model of the event. The few exceptions are probably explicable in terms of propagation anomalies. True, Knopoff and Randall looked into the possibility that body wave polarity distributions from deep focus earthquakes were somewhat removed from those of the simple model, but their evidence was not very strong. Almost all seismologists regard the model outlined above as an adequate description of the observed seismic source.

Nevertheless, there are some nagging problems. Plate tectonics explains shallow earthquakes as a manifestation of rubbing, slipping and cracking near plate boundaries and for such events the double-couple model of the source is entirely appropriate. But when plates descend beneath island arcs they presumably end up by being assimilated at a depth of about 700 km. During this descent into the mantle the lithospheric material has in all probability to undergo phase changes. The surrounding mantle is believed to contain large phase transitions at 400 km and 600 km depth at each of which physical properties suffer a change of several percent. Since there are earthquakes at all depths in the descending plate, the obvious question is whether deep events, although apparently double-couple in nature, contain any constituent, say, of compression that might be associated with an explosive phase change. Dziewonski and Gilbert's results indicate that this is a real possibility.

For several years the authors have been analysing the oscillations of the Earth which follow very large earthquakes. They and others have developed highly sophisticated methods for extracting signals at periods of up to 1 h from the hundred or so seismic stations around the world. As a result of this work the broad structure of the Earth's interior is known with some confidence. Until recently, however, the inversion process was simply from the observed eigenfrequencies to physical structure. There is more available, however—amplitude and phase of each eigenfrequency is accessible with careful processing. This permits inversion for the generating function, or source mechanism as a function of time. The solution from body waves for the source mechanism is a high frequency one, but free oscillations cover a lower frequency range. Does the earthquake look similar in appearance at lower frequency?

The answer is that it does not. Dziewonski and Gilbert look at two deep earthquakes in South America. They find, not surprisingly, that to the extent that their solutions can be interpreted as double couples they are in agreement with body wave solutions for the mechanisms. There seems, however, to be a substantial constituent of non-deviatoric stress released and this stress is dominantly compressive. Furthermore, the inversion indicates clearly that compressional energy is in both cases released from the vicinity of the event for at least 80 s prior to what is more conventionally called the earthquake—the time of double-couple radiation of high frequency energy. The release of stress seems to be very smooth and thus there is no record of high frequency signal from it.

This is a most exciting result. If deep focus earthquakes can have a compressive precursor then this may be a transition to a more dense phase. The authors point out that it is entirely possible that it is the heat generated by this transition which causes local melting and triggers the quake along a lubricated slip plane. They further suggest that there may be occasions when the compression proceeds without triggering a slip.

Care must be taken in contrasting deep and shallow quakes, for both of which precursory signals have now been recorded. In both cases it seems that earthquakes can occur through lubrication of the fault plane. For shallow events the lubrication seems to come through a long term change in the distribution of fluids in the source reigon. For deep events, if the authors' speculation is correct, the lubrication would arise from exothermic phase changes. Doubtless more will be heard of deep earthquakes in the next year or two.

D. D.

T cell Ig receptors— to be or not to be?

There are few subjects of investigation in cellular immunology which have attracted more controversy than the nature of the receptor for antigen on thymus-derived (T) lymphocytes. A good illustration of this is to be found in several recent articles, two of which by Roelants and colleagues, have appeared in *Nature* (**247**, 104 and 106; 1974).

The central question is whether T cells like B cells have surface bound immunoglobulin (Ig) receptors. Several groups of investigators have subjected lymphocytes to enzymatic radioiodination of their cell surface proteins, followed by disruption of the cell and precipitation by specific anti-immunoglobulin antisera. Using this method, Marchalonis *et al.* (*Transplant. Rev.*, 14, 1; 1973) claim to have detected and isolated significant quantities of surface Ig on T cells and on thymocytes. Their conclusion is that T and B cells have similar amounts of surface Ig. Vitetta *et al.* (*Transplant. Rev.*, **14**, 50; 1973), however, using the same method, could only detect minute quantities of membrane associated Ig on thymocytes or peripheral T cells. Furthermore, if such cell suspensions were first treated to eliminate small numbers of contaminating B cells, then the 'pure' T cell suspensions did not subsequently yield any Ig.

Using a similar method, Grey *et al.* (*J. exp. Med.*, **136**,, 1323; 1972) could not detect appreciable amounts of T cell Ig. Similarly, Lisowska-Bernstein *et al.* (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 2879; 1973) were unable to detect T cell Ig on thymocyte or cortisone-resistant thymocyte cell suspensions which had been pretreated so as to kill any contaminating B cells or IgM-containing plasma cells. Antigen-activated T cells were found by labelling experiments with radioactive leucine and methionine apparently to synthesise μ and light Ig chains, but again, this was probably due to contamination by IgM-containing plasma cells. The conclusion is that most if not all T cell Ig is adsorbed material, a conclusion made more plausible by the recent demonstration that activated T cells have Fc receptors which can recognise certain Ig molecules by means of their Fc portion and bind them readily when they are complexed to specific antigen (Yoshida and Andersson, *Scand. J. Immum.*, **1**, 401; 1972). Thus, of four laboratories, using very similar or identical techniques, three maintain that little or no Ig is made by T cells, and only a little more is sometimes displayed on the cell surface.

By contrast, Roelants, Rydén, Hägg and Loor (*Nature*, **247**, 106; 1974) conclude not only that T cells have antigen-specific Ig receptors, but that they are also actively synthesised by the cells carrying them. The system used by Roelants *et al.* to demonstrate T cell antigen-specific Ig receptors is indirect, involving inhibition af antigen binding between T cells and highly radioactive antigen (which is visualised and quantitated by radioautography) after pretreatment of the cells with a polyvalent rabbit anti-mouse Ig serum.

The anatomic origin of the antigen binding cells was determined by Roelants *et al.* by immunofluorescent techniques involving anti-θ serum as a T cell marker, or anti-Ig serum, as a B cell marker (T cells evidently have too few surface Ig molecules to be readily visualised by this second technique). Using this combined immunofluorescent-radioautographic technique, Roelants *et al.* demonstrated a small number of antigen-binding T cells to a synthetic antigen, which in turn could be inhibited by an anti-immunoglobulin serum, thus confirming previous observations (Roelants *et al.*, *J. exp. Med.*, **137**, 1060; 1973). But equally important, the authors go on to conclude that these surface Ig molecules are endogenously produced by the T cells themselves, and are not passively absorbed. The authors reasoned that if Ig molecules are passively absorbed, then after inducing their capping and internalisation by anti-immunoglobulin serum, the T cells should remain negative for cell surface Ig when incubated *in vitro* at 37°C. On the other hand, if endogenous synthesis of the Ig moieties is the rule, it should be possible to demonstrate the reappearance—and therefore 'recapping'—of the receptors. This second situation proved to be the case, and authors conclude their experiments demonstrate active synthesis of Ig receptors for antigen by T lymphocytes.

It should be pointed out that the validity of the interpretation is dependent on independent movement of cell surface components under the influence of capping stimuli. For example, it could be argued that T cell receptors which are intimately associated with passively-acquired surface Ig molecules could be 'co-capped' with the Ig molecules by the anti-Ig treatment. Roelants *et al.* say there is no such precedent for co-capping, but recently Schlesinger has cited unpublished observations from his laboratory indicating that H-2 antigens and C3 receptors on the cell surface of peritoneal macrophages can be co-capped (*Biomedicine*, **18**, 473; 1973).

It is difficult to reconcile the conflicting opinions which presently exist with regard to Ig receptor molecules on T cells. Fairly broad agreement is evolving that T cells do have surface Ig, but much less than B cells have; but whether these few molecules are passively acquired or are endogenously produced, now seems to be the centre of the controversy. Some of the experimental differences may eventually turn out to be related to the use of heterologous anti-immunoglobulin sera which all these experiments entail; although presumed to be 'monospecific', it is doubtful, even after rigorous absorption, whether such sera exist. A second factor which will have to be reckoned with is the part Fc receptors on certain types of T cells may be playing in the various experimental protocols. Basten *et al.* (*J. exp. Med.*, **135**, 610; 1972) have already cautioned others on the difficulties such receptors could cause in interpreting results of studies on antigen binding properties of B cells: this warning must now be extended to similar T-cell studies.

R. K.
A. D.

Growing hepatitis B virus

from our Medical Virology Correspondent

THE frustrating problem of propagating the elusive hepatitis viruses in tissue culture has been a major obstacle to defining the biophysical, biochemical and biological properties of these infectious agents and to the production of suitable vaccines. Hepatitis B antigen (Australia antigen), now generally believed to represent the outer protein coat of the virion, provides a convenient specific marker of infection with type B hepatitis virus. The availability of such a marker permitted some progress in tissue culture studies. Brighton and colleagues (*Nature new Biol.*, **232**, 57; 1971) for example, reported the induction of progressive non-cytocidal involvement of normal cellular components of monolayer cultures of human embryo liver cells after inoculation with a well-documented infective hepatitis B serum. Hepatitis B antigen was localised by immunofluorescence in the cytoplasm and in the nuclei of the cells. Smith and Francis (*Cancer Res.*, **32**, 1713; 1972) obtained similar results.

In subsequent studies, following the inoculation of supernatant fluid which had been passaged twice in culture, the outer coat (hepatitis B) antigen was demonstrated by immunofluorescence, using specific animal antiserum, in the cytoplasm of cultured liver cells. Only nuclear fluorescence was observed when human antibody was used (Zuckerman and Earl, *Vox Sang. Suppl.*, **24**, 123; 1973). Similar fluorescent changes were also noted by Coyne and her associates (*Proc. Soc. exp. Biol. Med.*, **138**, 1051; 1971 and London *et al.*, *Can. med. Ass. J.*, **106**, 480; 1972) in liver cells cultured from biopsies obtained from two patients with circulating hepatitis B antigen. In addition the antigen was detected by radioimmunoprecipitation in the supernatant fluid of a few cultures which had been serially passaged.

The morphological and functional capacity of cells maintained in organ culture offers a system for cultivating viruses which simulates conditions in the intact host. Such techniques have proved valuable for cultivating viruses which are difficult to grow in conventional cell monolayers. Cultured fragments of human inguinal lymph nodes were incubated by Jenson and colleagues (*Exp. molec. Path.*, **13**, 217; 1970) with serum containing hepatitis B antigen from a drug addict admitted to hospital with hepatitis. On the basis of serological tests and electron microscopy of the supernatant fluid and examination of ultrathin sections of the explants, it was concluded that some lymph node organ cultures may support the *in vitro* production of hepatitis B antigen. More recently, Zuckerman and associates (*Nature*, **236**, 78; 1972) reported that hepatitis B antigen may be produced in human embryonic liver organ cultures. A progressive increase in the titre of hepatitis B antigen, measured by several techniques and confirmed by immune electron microscopy, was shown with a limited number of sera, but only one successful passage of harvested material was accomplished.

Shikata (*Jap. J. exp. Med.*, **43**, 231; 1973) demonstrated hepatitis B antigen by immunofluorescence for 4 weeks in organ cultures of human foetal liver tissue grown in a Rose chamber. Specific fluorescence was also found in the cytoplasm of spindle-shaped cells growing out from the original liver tissue and it was considered that these fibroblast-like cells phagocytosed the antigen produced by the hepatocytes. The culture medium was not examined and serial passage was not attempted. Taylor and Kelen (*Proc. Can. Fed. Biol. Soc.*, **15**, Abst. 499; 1972) used liver organ and monolayer cultures from recently killed nonhuman primates. Fluid harvested 8 days after primary inoculation of Rhesus monkey liver organ culture with serum containing hepatitis B antigen was found to contain readily detectable antigen as well as a substantial increase in the proportion of the 42 nm (Dane) particles. Passage of harvested fluid was not achieved.

More exotic studies were lately described by Banatvala and Payne (*Lancet*, i, 1359; 1973), who found that sera from patients with hepatitis B infection and sera from some carriers of the antigen induced necrotic changes in the leaves of tobacco plants. Neutralisation tests and preheating the inoculated sera in a boiling water-bath inhibited the necrotic effects in the plants. But again transmission of the virus was not demonstrated.

Although many of these reports are encouraging and suggest that hepatitis B virus may be cultured with difficulty in mammalian cells, consistent serial passage of this virus had not been attained. Panouse-Perrin and her colleagues now report (*Biomed. Express*, **19**, 442; 1973) preliminary results of the cultivation of hepatitis B virus in a human diploid cell line derived from liver biopsies obtained from antigen-negative children undergoing surgery. Electron microscopy revealed the presence of 20 nm icosahedral particles with ninety-two capsomeres in the cell and in the supernatant fluid of the cultures after serial passage. After the fourth passage numerous clusters of capsids, empty icosahedrons about 20 nm in diameter, and electron dense particles measuring 25 to 27 nm were present. These particles are considered to be similar, if not identical, to the internal component or inner core of the 42 nm (Dane) particle (Almeida *et al.*, *Lancet*, ii, 1225; 1971). After fourteen passages cellular degeneration occurred and particles similar to the three morphological entities generally associated with hepatitis B antigen were visualised.

Serological tests, including radioimmunoassay, on supernatant fluid obtained after ultrasonic disintegration of the cultures proved negative for hepatitis B antigen; but precipitin lines were obtained by counter-immunoelectrophoresis when the fluids were tested against sera from patients in the early phase of convalescence from hepatitis B infection. The precipitin lines may have resulted from the interaction between the inner core antigen produced in the cultures and the antibody in serum of patients recovering from hepatitis (Almeida *et al.*, *loc cit*; and Hofnagle *et al.*, *Lancet*, ii, 869; 1973). If these observations can be confirmed and extended then a giant leap forward in hepatitis research has just taken place.

Genetic variation of histones

from our Molecular Genetics Correspondent

ONE of the most interesting observations to have been made in cell biology during the past couple of years is the conservation of amino acid sequences which seems to prevail among the histone proteins. Although only a small number of histones has been sequenced at present, one of the five fractions has an almost identical sequence in several species related only distantly in evolution; its extent of sequence conservation, in fact, seems to be the highest yet observed in any protein. Electrophoretic analysis of histone fractions suggests that conservation of the other four fractions may also be appreciable, although not as extensive as that of histone IV. The apparent homogeneity of sequence in each histone fraction has important consequences for the structure of the chromosomes and, a less fortunate consequence, means that investigations relying upon genetic variation may be rather difficult. In the November issue of the *Proceedings of the National Academy of Sciences*, however, Stout and Phillips (**70**, 3043; 1973) report the existence fo genetic variations in the lysine rich histone fraction I of maize (*Zea mays*).

The lysine rich group of histones is the only one of the five histone fractions that shows any internal variation; it seems to consist of several proteins whose sequences are identical in most of their length but vary at what are probably the amino acid residues present at only a small number of positions. Stout and Phillips have now shown that there may be a genetic polymorphism in these subfractions, with different groups of alleles coding for the lysine rich histones in different inbred lines of maize.

Slight differences in the electrophoretic mobilities of the subfractions of the lysine rich histones were first noticed when a line of maize carrying the *sticky* mutation—which produces sticky chromosomes at meiotic anaphase in pollen cells—was compared with another line lacking the mutation. The rationale for these experiments was that the *sticky* mutation might have as its molecular effect the alteration of properties of one of the chromosomal proteins. In genetic mapping experiments, the variation in one lysine rich histine subfraction (Ia) behaved as though coded by a single Mendelian-segregating gene, independent of the *sticky* locus. Another lysine rich subfraction showing genetic variation (Ic) seems to segregate independently of the locus controlling Ia variation.

In spite of the initial rationale for these experiments, the meiotic mutant *sticky*, and also another meiotic mutant *elongate*, do not seem to control a histone protein. But a comparison of several inbred maize lines showed that each exhibits a specific variation in its histone I subfraction pattern, consistent with earlier experiments which have shown that this fraction is the least conserved in evolution. The only other histone fraction which has been shown to comprise genetic variation is the unique fraction V, which replaces the lysine rich fraction I in avian erythrocytes. Greenaway and Murray (*Nature new Biol.*, **229**, 233; 1971) found that it has two subfractions under genetic control, one with a glutamine at position 15 and one with an arginine. These experiments however, did not show whether the segregating locus comprises only a single gene, as is the case in maize.

The demonstration that variation in lysine rich subfractions in maize may be due to single unlinked mendelian factors contrasts with conclusions derived from hybridisation with DNA of the messenger RNA fraction coding for the histones; these biochemical experiments have suggested that many genes—which in *Drosophila* may be clustered together—might code for each histone protein. But, of course, until the different histone I variations of maize have been sequenced, it is not possible to be certain that the gene controlling their segregation is the structural locus; although the lysine rich histones in general do not seem to suffer modification, until proved otherwise it remains possible that the segregating locus controls modification rather than synthesis *per se*.

The homogeneity of each histone fraction is most consistent with models which suppose that each protein is controlled by a single genetic locus. Although, of course, it is possible to construct models for the organisation of the genome which reconcile unifactorial genetic inheritance with gene multiplication, experiments with messenger RNAs purified for other gene products suggest that the presence in the genome of a single structural gene for each protein may be the more general phenomenon. The histone system at present poses more problems than it resolves, for the striking conservation of histone sequences in disparate organisms remains inexplicable; clearly, the whole sequence of (at least) histone IV is critical for the organism, variation of even a single amino acid being prohibited, which implies that there may be important similarities in chromosome structure in all eukaryotes. But although it is obvious that conservation of histone structures conveys an important message about the organisation of the genetic apparatus, the nature of this message remains elusive.

Diapause regulation by juvenile hormone

from our Insect Physiology Correspondent

When the hormonal control of moulting and metamorphosis in insects was defined about forty years ago it was suggested that the periodic arrest of growth, or reproduction, that occurs in many insect species and which is called 'diapause', was the result of an arrest of hormone secretion—since a state closely resembling natural diapause could be induced by the experimental exclusion of the growth hormones. Experiments on a wide range of insects have largely substantiated this idea. Sometimes it is the neurosecretory cells in the brain which are inactive; sometimes the prothoracic glands. In adult insects the failure of egg development during diapause results sometimes from a failure in the secretion of the juvenile hormone by the corpora allata, as in the potato beetle *Leptinotarsa*; sometimes from an arrest in the neurosecretory cells of the brain, as in overwintering mosquitoes.

But in this story the juvenile hormone has come to occupy a somewhat equivocal role. It was noted by the Japanese workers Fukaya and Mitsuhashi that in the caterpillar of the rice stem borer *Chilo*, exclusion of the corpus allatum induced the diapausing insects to resume their development. These authors therefore inferred that the juvenile hormone was acting as a 'diapause factor'.

Yin and Chippendale (*J. Insect Physiol.*, 19, 2403; 1973) have now reinvestigated this problem in another caterpillar, the southwestern corn borer *Diatraea*. It has been known for some years that in certain Lepidoptera the juvenile hormone experimentally applied may serve as a stimulus to the prothoracic gland to induce secretion of ecdysone and thus to bring about moulting in caterpillars deprived of the brain. This happens in *Diatraea*; and when sufficient juvenile hormone is present the insect undergoes a 'stationary moult', that is, it retains its larval form. Whereas, if only a very small amount of juvenile hormone is present the caterpillar changes to a pupa when it moults.

It has long been known that in Lepidoptera and other insects with a complete metamorphosis a high titre of juvenile hormone causes retention of larval characters, a lower titre signals the evocation of pupal characters, and in the absence of juvenile hormone adult characters appear. Yin and Chippendale produce good experimental evidence to support their novel hypothesis that the diapause state in this particular insect, *Diatraea*, is evoked by yet another defined level of the juvenile hormone titre, slightly lower than that required for a larval-larval ecdysis. In some of the diapause larvae the juvenile hormone titre is apparently high enough to activate the prothoracic gland and thus to promote stationary ecdyses from time to time.

Liquid crystals at Bangalore

from a Correspondent

To commemorate the twenty-fifth anniversary of the founding of the Raman Institute at Bangalore in India and to highlight the present role of the institute as a centre for liquid crystal research, Professor S. Chandrasekhar organised an international conference on liquid crystals from December 3 to 8. The conference was preceded by a week's winter school on liquid crystals led by four guest speakers—P. G. de Gennes (Collège de France, Paris), G. W. Gray (University of Hull) and G. H. Brown and A. Saupe (Liquid Crystal Institute, Kent State University, Ohio).

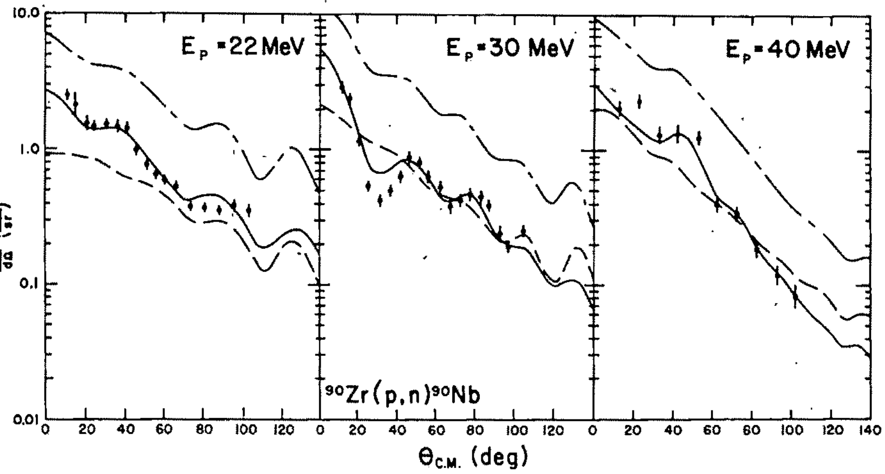
P. G. de Gennes opened the meeting by reviewing developments in the mixed elastic behaviour of smectics A phases. He dealt with continuous deformations, singular lines and dynamical aspects of smectic A to nematic transitions. This contribution and that by G. Durand (Orsay Liquid Crystal Group) on Rayleigh scattering in smectic and nematic materials reflected the high degree of interest in smectic phases and their polymorphism. A. de Vries (Kent State University) in his paper on X-ray studies of thermotropic liquid crystals then expounded his classification of smectic polymorphs based on X-ray diffraction patterns. This system of classification obviously does not satisfy everybody, and it is clear that there is not yet adequate structural information about the eight smectic polymorphs, for the existence of which experimental evidence has been proposed, to form the basis of a thoroughly satisfactory classification scheme. As yet uncategorised smectics probably exist, for example, the smectic phase of 4'-n-octyl-4-cyanobiphenyl described by Gray.

Areas of research which may contribute to solving the problems of smectic polymorphism are those concerned with elucidating molecular motion and diffusion in mesophases. Such investigations featured in presentations by B. J. Bulkin (Hunter College of the City University of New York) on infrared optical activity as a probe of nematic order, by J. M. Schnur (Naval Research Laboratory, Washington) on Raman spectroscopy of nematic and smectic mesophases, by R. Blinc (University of Ljubljana) on soft modes in nematic and smectic liquid crystals and by J. A. Janik (Institute of Nuclear Physics, Cracow) on estimations of rotational correlation times for nematic phases.

Several contributions were made by Chandrasekhar's liquid crystal group at Bangalore, and the following titles reflect the range of physical studies being made at this centre: determination of twist elastic constants of nematic liquid crystals; pressure induced mesomorphism; theory of reflection and transmission by cholesteric films; anomalous transmission in absorbing cholesteric liquid crystals; circular dichroism in absorbing mixtures of right and left handed cholesterics; role of permanent dipoles in nematic order; statistical thermodynamics of the nematic liquid crystal surface; new types of electrohydrodynamic flow patterns in nematic liquid crystals and nuclear magnetic resonance spectra of molecules oriented in thermotropic and lyotropic nematics. Several of these contributions represented joint investigations with the National Aeronautical Laboratory, Bangalore or the Bhabba Atomic Energy Centre, Bombay.

Other presentations of importance were given by Saupe on electrohydrodynamic instabilities—a valuable review illustrated by striking photomicrographs—by D. Demus (Martin Luther Universität, Halle) on calorimetric investigations of liquid crystals, by F. M. Leslie (University of Strathclyde) on a theoretical analysis of distorted twisted orientation patterns in nematics and by E. J. Ambrose (Chester Beatty Research Institute, London) on the liquid crystalline properties of biological microfilaments.

Present interest in liquid crystals for electro-optical displays was reflected in the contributions by J. Billard (Collège de France and Université de Lille), who illustrated how eutectic compositions can be calculated for mixtures of mesogens, and by Gray who described further developments relating to the colourless, stable, room temperature smectics, nematics and cholesterics provided by 4′-n-alkyl- and 4′-n-alkoxy-4-cyanobiphenyls. Such compounds and their mixture (including those incorporating *p*-terphenyl analogues) provide excellent materials for twisted nematics and cholesteric-nematic phase change displays. S. Kobayashi (Saitama Institute of Physical and Chemical Research, Japan) also discussed off-90° twisted nematic films for use as voltage dependent colour controlled devices and orthogonal twisted nematic films, with coloured polarisers, for producing a range of coloured displays.



Differential cross sections for the (p,n) reactions to the isobaric analogue states compared with calculations with the one-step process only (—·—), the two-step process only (- - -) and the one-step and two-step processes combined (——).

Two-step nuclear reactions

from our Nuclear Theory Correspondent

THERE has recently developed a growing awareness of the importance of multistep processes in nuclear reactions. These are of two types: reactions involving pre-excitation of the target nucleus or post-excitation of the residual nucleus by inelastic scattering and reactions involving sequential transfer. If the direct process is inhibited in any way, the multistep processes can account for a significant fraction of the measured cross section, so that any conclusions concerning the structure of the participating nuclei based on the assumption that only the direct one-step process contributes may be gravely in error. Among the many possible multistep processes the two-step ones are the easiest to calculate, and detailed theories have now been programmed for electronic computation.

A relatively simple example of the importance of including two-step processes is provided by the (p,n) reaction to the isobaric analogue state of the target nucleus. This final state has essentially the same structure as the ground state of the target apart from a reversed isospin vector for one of the target nucleons: a neutron is replaced by a proton in the same shell-model state. On a simple view the reaction thus proceeds entirely by the isospin term in the nucleon-nucleus optical potential. The total wave function is thus expressible as a sum of the wavefunction in the elastic channel and that in the (p,n) or quasi-elastic channel. Use of the Schrödinger equation with an isospin-dependent potential that can cause transitions from the elastic to the quasi-elastic channel gives a pair of coupled differential equations (called the Lane equations) for the two wave functions. These can be solved to give the observable cross sections in terms of the parameters of the potentials used. It is then possible by comparing calculations with the experimental data to obtain the best values of the parameters of the potential, in particular of the isospin term. Such analyses have been moderately successful in that with suitable choice of parameters it is possible to fit the data fairly well, and to obtain an estimate of the isospin potential.

These analyses assume that the reaction is a direct one-step process from the incident to the final state. It is, however, possible that the reaction can take place in two or more stages, and this can be investigated using a microscopic model of the interaction in which the contributions of all nucleons outside closed shells are explicitly included.

This model has recently been used by Rickertsen and Kunz (*Phys. Lett.*, **47B**, 11; 1973) to study the contribution to the quasi-elastic cross section due to successive particle transfer: instead of direct (p,n), there is a (p,d) pickup followed by (d,n) stripping, leading to the same final state as before. The formalism for this process is much more complicated than for the one-step process, but it has been worked out to give a set of coupled differential equations that can be solved on a computer.

In these microscopic interactions the essential parameters are those of the effective nucleon-nucleon interaction, expressed as a sum of central and isospin terms, together with those of the potentials describing the distortion of the incident and outgoing waves and the shell model used for the initial and final nuclei.

Rickertsen and Kunz have made a series of analyses of (p,n) quasi-elastic cross sections using both the one-step process on its own and then the one-step with the addition of the two-step. It was found, as shown in the figure, that the one-step alone is not always very successful, even if the absolute magnitude is adjusted by altering the strength of the isospin term but that with the addition of the two-step process the agreement is very satisfactory. It is notable that the one-step contribution on its own gives a cross section greater than that observed, so there is considerable destructive interference when the two-step process is included as well.

It is also found that the strength of the isospin term in the effective nucleon-nucleon interaction varies over a wide range from about 15 MeV to 33 MeV if the data are analysed with the one-step process alone, whereas if the two-step process is included the values of the strength cluster much more closely around a mean value of about 29 MeV. The contributions of the two-step process differ from nucleus to nucleus depending on their individual shell structure and they are such as to account for the data, when combined with the one-step process, with parameter values having a more uniform behaviour from nucleus to nucleus.

Thus both the angular distributions and the absolute magnitudes of the (p,n) quasi-elastic cross sections are much better accounted for by the inclusion of the two-step process. It is physically very plausible and gives a more coherent account of the experimental data. The contribution of a two-step process involving pre- or post-excitation of the target or residual nucleus by inelastic scattering was also calculated and found to be quite small.

The possibility of such two-step contributions to nuclear reactions must therefore be carefully considered, and a full calculation made if one is to obtain a detailed and accurate account of the data.

Upper mantle model variations

from our Geomagnetism Correspondent

As the newly-formed oceanic lithosphere spreads away from its ridge source it gradually cools and contracts; and the elevated, rugged topography characteristic of the ridge ultimately gives way to the lower, smoother surface beneath the sediments of the ocean basin. If the cooling, contracting lithosphere were to move over a laterally uniform asthenosphere, the only other factor governing the ridge shape and dimensions would be the spreading rate. Thus several workers, beginning with McKenzie (*J. geophys. Res.*, **72**, 6261; 1967), have constructed thermal models involving the structure of the lithosphere across ocean ridges in which particular attention has been paid to the effect of spreading rate on the topographic profile. In all such models the spreading rate was found to affect principally the horizontal scale of the topography, with an increase in spreading rate producing a lengthened section but almost no variation in vertical dimensions. But as Haigh (*Geophys. J.*, **33**, 405; 1973) has now pointed out with reference to the North Atlantic, although the differences in horizontal scale predicted by previous thermal models exist, there are also variations of ridge and basin structure with latitude which cannot be attributed to differences in spreading rate. He thus concludes that the basic premise of a uniform asthenosphere is wrong, and that instead the temperature at a given depth in the asthenosphere must vary laterally.

The latitudinal variations which require explanation have been known in general terms for some time, the particular data now analysed by Haigh being derived from Atlantic bathymetric and gravimetric profiles obtained as long ago as 1964–1965. From these it is clear, for example, that between low latitudes and Iceland the depth at which the topographic profiles across the Atlantic ridge system become level in the ocean basins decreases northwards from 5.5 km at 30°N to 3.0 km and 60°N. Because the ocean basin level is not reached at the same distance from the ridge throughout the whole latitude range, there may be a suspicion that the variation of basin depth with latitude arises from differences in lithospheric age. No such explanation is possible here, however, because all the ages involved are greater than 60 million years; and as Sclater *et al.* (*J. geophys. Res.*, **76**, 7888; 1971) have shown, changes in elevation beyond this age are negligible. Nor can the marked northward decrease in basin to ridge crest elevation between the Azores and Iceland (the region studied in detail by Haigh) be explained away quite so simply. On the other hand, spreading effects are significant when it comes to ridge cross-sectional area. Even so, when the effects of variable spreading rate on horizontal scale are adjusted throughout to the spreading rate at 43°N, there is still found to be a marked decrease in ridge cross-section area northwards from the Azores.

To investigate the causes of these variations Haigh has constructed a model in which, in common with previous thermal models, the lithosphere is regarded as a slab of uniform thickness resting isostatically on an asthenosphere assumed to be at constant temperature across the ridge. As new material at this temperature is added gradually to one end of the slab, the slab increases in length so that a point on the lithosphere moves away from the hot edge at a rate governed by the rate at which new material is added. Solution of the relevant differential equations in conjunction with assumed values of the physical parameters involved then gives the temperature distribution in the lithospheric slab for any given slab thickness and composition. But in order to obtain an elevation profile from the temperature distribution it is necessary to know the density of the lithospheric material at the appropriate temperatures and pressures—and this involves all the usual problems in dealing with a body whose composition is unobtainable by direct sampling.

Haigh's approach has been to asssume that the upper mantle comprises basalt and dunite, with or without water, and either in the ratio 1:3 as suggested by Ringwood (*J. geophys. Res.*, **67**, 857; 1962) in his 'pyrolite' model or in the ratio 1:1 as proposed by Press (*Science, N.Y.*, **165**, 174; 1969). The end result is then a series of calculated ocean ridge profiles (elevation-distance graphs out to 900 km form the ridge) for the two different basalt-dunite compositions, with or without the involvement of water, and for various lithospheric thicknesses. In addition, to account for possible heat transfer by radiation, a futher variable in the form of a conductivity increase above 800°C has been introduced.

The general conclusion derived from a comparison of the calculated and observed profiles was that many models give too great a ridge elevation unless the allowed ranges of lithospheric thickness and composition are limited. More specifically, a composition involving a basalt-dunite ratio of 1:3 was found to be more likely than one with a ratio of 1:1; and the mantle must contain a small amount of water in order to allow the decoupling between the lithosphere and asthenosphere to occur at a lower temperature than is necessary for a dry model. In addition, models involving a conductivity increase above 800°C were in poor agreement with observation, having too much elevation at the ridge axis and not enough on the flanks. And, finally, raised temperatures in the asthenosphere were found to give an upward migration of phase boundaries, resulting in a thinner lithosphere and thus a reduction of ridge cross section and elevation.

In the context of the observed variations with latitude between the Azores and Iceland the last of these conclusions is particularly significant, for it implies an explanation. Haigh's next step was thus to compare the North Atlantic topographic profiles with profiles calculated for a range of lithospheric thicknesses. From a least squares fit between each observed profile and the calculated profiles with different thicknesses, it became clear that the lithosphere thins from 85 km at 43°N to 64 km at 61°N (although these thicknesses increase by about 10 km on average if the presence of sediments is taken into account). In view of the conclusion that a thinned lithosphere is associated with raised temperatures in the asthenosphere, Haigh thus suggests that the temperature at a given depth in the asthenosphere gradually increases northwards from the Azores to Iceland. He is also able to show that such a lateral temperature variation in both the asthenosphere and lithosphere can explain the northward rise of the whole basin and ridge system with respect to sea level, and not just the variation of those parameters such as ridge cross section and elevation which are measured with respect to the ocean basin level.

In thinking about a northward temperature increase towards Iceland it is tempting to see Iceland as the region of maximum temperature and thus as either the site of an uprising mantle plume as suggested by Morgan (*Nature*, **230**, 42; 1971) or of a convective overturn as suggested by Bott (in *Implications of Continental Drift to the Earth Sciences*, Academic Press, 1973). If Iceland really is the temperature focus, the northward variations between the Azores and Iceland should be mirrored by similar variations southward between the Arctic and Iceland. At present there are few data from north of Iceland, and so little can be said about this except that Haigh claims some support for the dimensional increase in the ridge to the north in the work of Johnson and Heezen (*Deep-Sea Res.*, **14**, 755; 1967). For the time being, Haigh is obviously unwilling to be drawn into a detailed argument over mantle plumes, and rests content with the comment that his model is consistent with uniquely higher temperatures beneath Iceland. But he emphasises that his general conclusions, if valid, should be applicable to other ridge systems.

Flux of Gases across the Air-Sea Interface

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This article describes the use of a two-layer model to estimate the flux of various gases across the air-sea interface.

In the study of gas exchange between air and water, the interface between the two phases is often considered as a two-layer (film) system[1,2]. The main body of each fluid is assumed to be well mixed; the main resistance to gas transport coming from the gas and liquid phase interfacial layers, across which the exchanging gases transfer by molecular processes (Fig. 1).

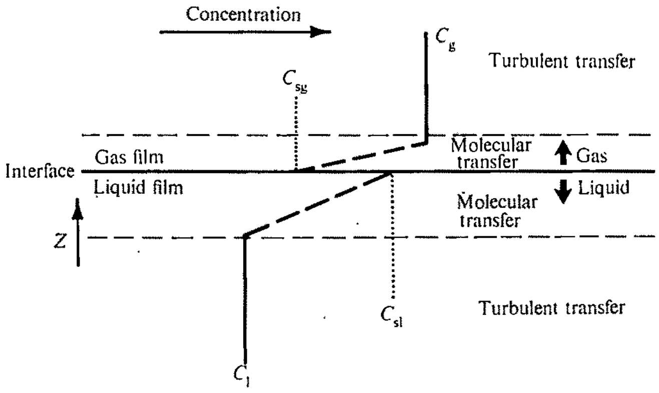

Fig. 1 Two-layer model of a gas–liquid interface.

That this simple model adequately describes processes at the interface must be open to question. The idea of such discontinuities close to the interface is physically unrealistic and, even under given conditions of turbulence in both phases, the layer thicknesses will vary both spatially and temporally. In spite of these difficulties, the film model is useful in visualising processes at the interface and for simplifying theoretical calculations of gas exchange rates. Furthermore, predictions based on the film approach often show little or no difference from those derived using more sophisticated models[2].

Since transport through the layer system is by molecular diffusion, Fick's first law in the one-dimensional form (with z as the vertical direction) is applicable.

$$F = -D\,\partial c/\partial z \tag{1}$$

where F is the flux of gas through layer; D the coefficient of molecular diffusion of gas in layer material; and c the gas concentration.

The more usual form of equation (1) for gas exchange studies is

$$F = k\Delta c \tag{2}$$

where Δc is the concentration difference across the layer (thickness z) and

$$k = D/z \tag{3}$$

k is called the exchange constant and has dimensions of velocity; it is a measure of the flux of gas per unit concentration gradient. The value of k depends on many factors, of which the degree of turbulence in the fluids on both sides of the interface and the chemical reactivity of the gas are among the most important. The reciprocal of the exchange constant is a measure of the 'resistance' to gas transfer, and has dimensions of $[T]$ $[L]^{-1}$. The total resistance to the exchange of any gas will be due to a combination of the resistances of the gas and liquid phases. Any surface contaminants will contribute to the resistance of the phase in which they occur.

Applying equation (2) to the two-layer situation shown in Fig. 1, and assuming that transport of gas across the interface is a steady state process, it follows that

$$F = k_g(c_g - c_{sg}) = k_l(C_{sl} - C_l) \tag{4}$$

where k_g and k_l are the exchange constants for the gas and liquid phases, respectively.

If the exchanging gas obeys Henry's law, then

$$c_{sg} = HC_{sl} \tag{5}$$

where H (the Henry's law constant) = [equilibrium concentration in gas phase (g per cm^3 of air)]/[equilibrium concentration of unionised dissolved gas in liquid phase (g per cm^3 of water)].

Eliminating c_{sg} and C_{sl} between equations (4) and (5),

$$F = (c_g - HC_l)/(1/k_g + H/k_l) = (c_g/H - C_l)/(1/k_l + 1/Hk_g) \tag{6}$$

which can be written as

$$F = K_g(c_g - HC_l) = K_l(c_g/H - C_l) \tag{7}$$

where

$$1/K_g = 1/k_g + H/k_l \tag{8}$$

and

$$1/K_l = 1/k_l + 1/Hk_g \tag{9}$$

Hence, the total resistance (R) expressed on either a gas phase ($1/K_g$) or a liquid phase ($1/K_l$) basis, depends on the exchange constants of the individual phases and the value of the Henry's law constant for the gas concerned.

For convenience in using equation (9), $1/k_l$ may be written as r_l, and $1/Hk_g$ as r_g,

$$R_l = r_l + r_g \tag{10}$$

The numerical values of r_l and r_g for any gas indicate the relative importance of gas and liquid phase resistance for exchange of that particular gas. Except in special cases, it is difficult to measure individual resistances, and most field and laboratory studies have measured only the total resistance. As will be shown, however, for many gases the resistance of one phase tends to predominate. In such cases the total measured resistance is, for all practical purposes, numerically equal to the individual resistance of the rate controlling phase.

Factors Affecting Resistance

Gas phase resistance (r_g). In common with evaporation-condensation of any appreciably pure liquid, the liquid phase resistance should be negligible for water molecules crossing the air–sea interface[3]. This means that for water $r_l = 0$, and exchange of H_2O across the interface will be controlled by processes in the gas phase. In this case the total measured resistance (R) will be numerically the same as the resistance of the gas phase (r_g). Laboratory studies indicate that the value of $k_{g(H_2O)}$ increases linearly with wind velocity[4]. Using the field data of Schooley[5], a mean value for $k_{g(H_2O)}$, applicable to the sea surface, of about 3,000 cm h^{-1}, may be derived. This figure, along with appropriate values for the Henry's law constant, can be substituted into equations such as (8) and (9) in order to split observed values of $K(=1/R_t)$ into their liquid and gas phase components.

Liquid phase resistance (r_l). A number of techniques have been used to determine K_l *in situ* at the sea surface. The results of these experiments have been summarised by Liss[4] and Broecker and Peng[6] and a mean value of $K_l = 20$ cm h^{-1} seems reasonable.

For O_2, $H \sim 30$, $k_{g(O_2)} \sim k_{g(H_2O)} = 3{,}000$ cm h^{-1}. Substituting these, and a value for $K_{l(O_2)}$ of 20 cm h^{-1} into equation (9), shows that $r_l \gg r_g$ and so $k_{l(O_2)} = 20$ cm h^{-1}, that is, for O_2 all resistance to transfer across the interface occurs in the liquid phase. The same is true for all sparingly soluble gases which are not chemically reactive, for example N_2, and the noble gases.

In the case of a chemically reactive gas, transport across the liquid film may be more complex than by straightforward molecular diffusion. For example, with CO_2 as well as the usual concentration gradient for gas molecules in physical solution, at a suitable pH there is also a similar gradient for HCO_3^- and CO_3^{2-} ions. It has been shown in laboratory experiments[4,7] that for $p\text{H} > 5$, and under moderately calm conditions, the ionic species gradient can contribute significantly to the exchange of CO_2 across the interface. Hoover and Berkshire[7] have used a one-layer (liquid) film model to derive the following equation which successfully predicts the exchange enhancements for CO_2 found in laboratory experiments.

$$\alpha = [\tau(\sqrt{(K_0^1 \tau/D)} \cdot z_l)]/[(\tau - 1)(\sqrt{(K_0^1 \tau/D)} \cdot z_l) + \tanh(\sqrt{(K_0^1 \tau/D)} \cdot z_l)] \quad (11)$$

where α is the fractional increase in the exchange constant due to chemical reactivity; τ the ratio of total to ionic forms of inorganic carbon; K_0^1 the hydration rate constant for CO_2 in water; and z_l the thickness of liquid film.

The degree of exchange enhancement for CO_2 depends on the pH (which determines τ), the thickness of the interfacial liquid layer and the hydration rate constant. It has been shown in laboratory experiments that K_l increases approximately as the wind velocity squared[4,8,9], and that enhancement of $K_{l(CO_2)}$ over $K_{l(O_2)}$ can occur at low wind speed[4]. Under more turbulent conditions the thickness of the liquid film decreases to such an extent that re-equilibration of ionic species becomes much less important and is overriden by molecular diffusion. This is the situation at the sea surface, where conditions are generally too rough for enhancement of CO_2 transfer by more than a few per cent[4,7,10]. In the case of gases with larger hydration rate constants, however, such enhancement may become much more important (see SO_2 later).

We have so far attempted to establish that values for the gas and liquid phase exchange constants appropriate to the sea surface are

$$k_{g(H_2O)} = 3{,}000 \text{ cm h}^{-1}; \; k_l = 20 \text{ cm h}^{-1}$$

In order to obtain the k_g value for gases other than water, the value of $k_{g(H_2O)}$ must be multiplied by the ratio of the square roots of the molecular weights of H_2O and the other gas. The value for k_l is based largely on measurements of CO_2 exchange and is probably valid for gases of molecular weight 40 ± 25. For gases outside this range k_l should be multiplied by the ratio of the square roots of the molecular weights of CO_2 and the other gas. For a chemically reactive gas it is probable that k_l may be corrected for chemical enhancement by multiplying it by the value of α derived using equation (11).

In Table 1 values of k_g and k_l, plus a knowledge of H, are used in conjunction with equations (8) and (9) to derive overall exchange constants (K_g or K_l) for each of a number of gases. In subsequent sections this value of K, together with the observed concentration difference of each gas across the air–sea interface, will be substituted into equation (2) to calculate the flux of that gas across the interface.

For all gases considered, other than SO_2, liquid phase resistance controls the exchange ($r_l \gg r_g$). For SO_2 gas phase

Table 1 Calculation of Overall Exchange Constants for a Number of Gases crossing the Air–Sea Interface

Gas	k_g (cm h^{-1})*	α†	k_l (cm h^{-1})‡	H	r_g (h cm^{-1})	r_l (h cm^{-1})	r_g/r_l	K (cm h^{-1})§
SO_2	1,600	1,721	34,420	3.8×10^{-2} (ref. 11)	6.3×10^{-4}	1.1×10^{-6}	573	1.6×10^3 (g)
N_2O	1,900	1.0	20	1.6	3.3×10^{-4}	5.0×10^{-2}	6.6×10^{-3}	20 (l)
CO	2,400	1.0	20	50 (ref. 12)	8.3×10^{-6}	5.0×10^{-2}	1.7×10^{-4}	20 (l)
CH_4	3,180	1.0	20	42 (ref. 13)	7.5×10^{-6}	5.0×10^{-2}	1.5×10^{-4}	20 (l)
CCl_4	1,030	1.0	10.7	1.08¶	9.0×10^{-4}	9.3×10^{-2}	9.7×10^{-3}	10.7 (l)
CCl_3F	1,085	1.0	11.3	5¶	1.8×10^{-4}	8.8×10^{-2}	2.0×10^{-3}	11.3 (l)
MeI	1,070	1.0	11.1	0.24¶	3.9×10^{-3}	9.0×10^{-2}	4.3×10^{-2}	10.6 (l)
$(Me)_2S$	1,620	1.0	20	0.3 (ref. 14)	2.1×10^{-3}	5.0×10^{-2}	4.2×10^{-2}	19.2 (l)

* All values corrected for molecular weight.

† All gases, other than SO_2, assumed to be unreactive ($\alpha = 1.0$). α_{SO_2} calculated using equation (11) and τ-1 (at pH 8.0) $= 8.032 \times 10^{-8}$, $K_0^1 = 3.4 \times 10^6$ s^{-1} (ref. 15), $D = 1.8 \times 10^{-5}$ cm^2 s^{-1}, $z_l = D_{(O_2)}/k_{l(O_2)} = 3.96 \times 10^{-3}$ cm.

‡ Values for CCl_4, CCl_3F and MeI corrected for molecular weight.

§ Overall exchange constant, $K = (r_g + r_l)^{-1}$, expressed on either a gas (g) or liquid (l) phase concentration basis.

¶ J. E. Lovelock, personal communication.

resistance is all important ($r_g \gg r_l$). This is in part due to the high solubility of SO_2, but also because of its extremely rapid hydration reaction. A fuller discussion of this point and values of the ratio r_g/r_l at *p*H values other than 8.0 may be found in refs 16 and 17. Exchange of other gases with high solubilities and/or rapid aqueous phase chemistry (for example, NH_3, SO_3 and HCl) is probably also controlled by gas phase resistance.

Flux of Various Gases

Sulphur dioxide (SO_2). In order to substitute the value for $K_{g(SO_2)}$ given in Table 1 (1.6×10^3 cm h^{-1} = 0.44 cm s^{-1}) in equation (2) the mean concentration of SO_2 in oceanic air is required. Junge[18] found values in Hawaii and Florida of 1 and 3 μg m^{-3} respectively. As the measurements were made on the coast and the winds were predominantly from the sea, Junge concludes that these values are fairly representative of marine conditions. Of the measurements made at sea, those for the North Sea[19] are in the range 3 to 9 μg m^{-3}, and for the North Atlantic <1 to 4 μg m^{-3} (ref. 20). These observations seem to point to a value of about 3 μg m^{-3} (3×10^{-12} g cm^{-3}) for the mean SO_2 level in air over the oceans.

Because of the chemical reactivity of SO_2 (rapid hydration and oxidation), it seems reasonable to assume that the SO_2 concentration in oceanic surface waters is zero and that the oceans act as a perfect sink for SO_2.

Substitution in equation (2) gives $F_{(SO_2)} = 4.2 \times 10^{-5}$ g cm^{-2} yr^{-1}. If this flux occurs over the whole area of the oceans (3.6×10^{18} cm^2), the total flux of SO_2 from atmosphere to oceans is 1.5×10^{14} g yr^{-1}. This figure is close to estimates of the total world input of SO_2 to the atmosphere from the burning of fossil fuels (10^{14} g yr^{-1}, ref. 21).

There is good agreement between our value for the air–sea SO_2 flux and the estimates of Eriksson[22] (2×10^{14} g yr^{-1}) and Robinson and Robbins[23] (0.5×10^{14} g yr^{-1}). Our estimate is substantially lower than that of Spedding[24] (9.6×10^{14} g yr^{-1}). This discrepancy arises from Spedding's use of a rather large value for $K_{g(SO_2)}$ (1.4 cm s^{-1}) and an atmospheric SO_2 concentration of 6 μg m^{-3}.

Nitrous oxide (N_2O). Junge and Hahn[25], from measurements in the North Atlantic, found a mean N_2O air concentration of 0.25 p.p.m. (by volume) and an average concentration in the water (expressed on a gas phase basis) of 0.4 p.p.m. This gives a concentration difference of 0.15 p.p.m., which is equivalent to 0.09×10^{-6} cm^3 N_2O per cm^3 of water on a liquid phase concentration basis. Hence the concentration gradient driving the flux of N_2O from the water to the air is 1.8×10^{-10} g cm^{-3}. Substitution of this concentration difference and $K_{l(N_2O)} = 20$ cm h^{-1} in equation (2) gives $F_{(N_2O)} = 3.2 \times 10^5$ g cm^{-2} yr^{-1}. Assuming this flux takes place all over the oceans, the total flux of N_2O from sea to atmosphere is 1.2×10^{14} g yr^{-1}.

This sea to air flux of N_2O may be of very considerable importance in the oceanic nitrogen budget. The amount of fixed nitrogen entering the oceans from river inflow and rain is about 8×10^{13} g yr^{-1}, of which approximately 10% (9×10^{12} g yr^{-1}) is removed to marine sediments in the form of organic nitrogen compounds[26]. The flux of N_2O calculated above is equivalent to 7.6×10^{13} g N yr^{-1}, which is large enough to account for all of the nitrogen inflow to the oceans not removed to the sediments.

Carbon monoxide (CO). From measurements in the Pacific and North Atlantic, Lamontagne *et al.*[27] gives a value of 0.13 p.p.m. for the marine aerial concentration of CO. This is in agreement with estimates by other authors which fall in the range 0.03 to 0.3 p.p.m. Concentrations in the water are subject to diurnal cycling, varying between 2×10^{-8} and 13×10^{-8} cm^3 CO per cm^3 of water[27]. From measurements in the North Atlantic, Seiler (cited in ref. 28) obtains water values in the range 2×10^{-8} to 10×10^{-8}. We will use a mean surface water concentration of 6×10^{-8}. The mean aerial concentration expressed on a liquid phase basis is 2.6×10^{-9} cm^3 CO per cm^3 of water, and so the concentration difference sea–air is 5.7×10^{-8} (that is, surface seawater is twenty-three times supersaturated). This is equivalent to 7.1×10^{-11} g CO cm^{-3}. Substitution in equation (2), along with $K_{l(CO)} = 20$ cm h^{-1}, gives $F_{(CO)} = 1.2 \times 10^{-5}$ g cm^{-2} yr^{-1}. Provided the value used for the mean seawater concentration of CO is applicable to the surface oceans as a whole, the total flux of CO from sea to air is 4.3×10^{13} g yr^{-1}. The magnitude of this calculated flux is about 20% of the amount of CO injected into the atmosphere by human activities (2×10^{14} g yr^{-1}) (ref. 28).

Methane (CH_4). The mean atmospheric concentration of CH_4 over the North Atlantic and North Pacific was found by Lamontagne *et al.*[27] to be 1.4 p.p.m. (by volume). This agrees well with other values found and estimated for the marine atmosphere which fall in the range 1.1 to 1.6 p.p.m. Lamontagne *et al.*[27] measured methane concentrations in surface seawaters at the same time as the air samples were taken, and obtained a mean water value of 4.0×10^{-8} cm^3 CH_4 per cm^3 of water. Expressing the atmospheric CH_4 levels on a liquid phase basis, 1.4 p.p.m. = 3.3×10^{-8} cm^3 CH_4 per cm^3 of water, and comparing this with the mean surface water CH_4 concentration of 4.0×10^{-8}, indicates that the water is only about 20% supersaturated with respect to CH_4 in the air. The concentration difference sea–air is 0.7×10^{-8} cm^3 CH_4 per cm^3 of water (= 5.0×10^{-12} g CH_4 per cm^3 of water). Substitution in equation (2), along with $K_{l(CH_4)} = 20$ cm h^{-1} gives $F_{(CH_4)} = 8.8 \times 10^{-7}$ g cm^{-2} yr^{-1}. If this flux occurs over the whole of the ocean surface, the total flux of CH_4 from sea to air is 3.2×10^{12} g yr^{-1}.

The input of methane from the ocean to the atmosphere is more than an order of magnitude less than the flux of carbon monoxide calculated in the previous section. This arises because of the rather small degree of supersaturation of CH_4 at the ocean surface.

Carbon tetrachloride (CCl_4). Lovelock *et al.*[29] found a mean concentration of CCl_4 in the marine atmosphere over the Atlantic of 71.2×10^{-6} p.p.m. (by volume). Expressed on a liquid phase basis this is 66×10^{-12} cm^3 CCl_4 cm^{-3}. The mean surface water concentration found for samples collected at the same time as the air samples was 60×10^{-12} cm^3 CCl_4 cm^{-3}. Thus the air–sea concentration difference is 6×10^{-12} cm^3 CCl_4 cm^{-3}, which corresponds to 4.1×10^{-14} g CCl_4 cm^{-3}. Substitution in equation (2) along with $K_{l(CCl_4)} = 10.7$ cm h^{-1} gives $F_{(CCl_4)} = 3.9 \times 10^{-9}$ g cm^{-2} yr^{-1}. The total flux of CCl_4 from air to sea is 1.4×10^{10} g yr^{-1}.

The total industrial production of carbon tetrachloride is about 10^{12} g yr^{-1}, of which about 95% is converted to CCl_3F and similar compounds. Hence the maximum amount of CCl_4 which can escape to the air is 5×10^{10} g yr^{-1} (ref. 29). Our calculated total flux of CCl_4 from the atmosphere to the oceans is about 30% of this figure.

Trichlorofluoromethane (Freon-11, CCl_3F). Lovelock *et al.*[29] have found a mean concentration of CCl_3F in the marine atmosphere over the Atlantic of 50×10^{-6} p.p.m. (by volume) or, expressed on a liquid phase basis, 10×10^{-12} cm^3 CCl_3F per cm^3 of water. The mean surface water concentration measured simultaneously was 7.6×10^{-12} cm^3 CCl_3F cm^{-3}. The concentration difference air—water = 2.4×10^{-12} cm^3 CCl_3F cm^{-3} = 1.5×10^{-14} g cm^{-3}. Substitution in equation (2) along with $K_{l\,(CCl_3F)} = 11.3$ cm h^{-1} gives $F_{(CCl_3F)} = 1.5 \times 10^{-9}$ g cm^{-2} yr^{-1}. The air–sea flux of CCl_3F over the whole of the oceans is 5.4×10^9 g yr^{-1}.

United States production of CCl_3F is 0.09×10^{12} g yr^{-1} (ref. 21). From this a total world production of about 0.3×10^{12} g yr^{-1} seems reasonable. As there are no known natural sources for this compound the calculated flux from the atmosphere to the ocean seems to be about 2% of the world production.

Iodomethane (MeI). Lovelock *et al.*[29] found a mean atmospheric concentration of iodomethane over the Atlantic of 1.2×10^{-6} p.p.m. (by volume). Expressed on a liquid phase basis this is 5.0×10^{-12} cm^3 MeI per cm^3 of water. The mean surface water concentration was 135×10^{-12} cm^3 MeI cm^{-3}. Hence the sea–air concentration difference $= 130 \times 10^{-12}$ cm^3 MeI cm$^{-3} = 8.2 \times 10^{-13}$ g MeI cm^{-3}. Substitution in equation (2) along with $K_{1(\mathrm{MeI})} = 10.6$ cm h^{-1} gives $F_{(\mathrm{MeI})} = 7.6 \times 10^{-8}$ g cm^{-2} yr^{-1} and the total flux of MeI from oceans to atmosphere $= 2.7 \times 10^{11}$ g yr^{-1}.

It is well known that the I/Cl ratio in freshwater environments is much higher than the same ratio in seawater. One way to explain this is to assume that there is a preferential transfer of I relative to Cl across the sea–air interface. Miyake and Tsunogai[30] propose evaporation of I_2 molecules from the sea surface as the transfer mechanism. Such an explanation seems doubtful as no one has yet detected I_2 in seawater samples. Further, even if I_2 were found near the interface it would be likely to react with dissolved organic molecules which exist in high concentration at the sea surface[31].

Table 2 Calculated Fluxes of Various Gases crossing the Air–Sea Interface

Gas	SO_2	N_2O	CO	CH_4	CCl_4	CCl_3F	MeI	$(Me)_2S$
Total oceanic flux g yr^{-1}	1.5×10^{14}	1.2×10^{14}	4.3×10^{13}	3.2×10^{12}	1.4×10^{10}	5.4×10^{9}	2.7×10^{11}	7.2×10^{12}
	(−)	(+)	(+)	(+)	(−)	(−)	(+)	(+)

+, Sea→air; −, air→sea.

Miyake and Tsunogai[30] have calculated that in order to balance the iodine cycle on the Earth, 5×10^{11} g I yr^{-1} must cross the sea–air interface. The calculation given above indicates that the flux of iodomethane from the oceans to the atmosphere is about 2.7×10^{11} g yr^{-1}, which is equivalent to 2.4×10^{11} g I yr^{-1}. This is approximately half the total amount of iodine needed to balance the budget for this total amount of iodine needed to balance the budget for this element. In our view iodomethane is a much more likely vehicle than I_2 for the transfer of iodine from the ocean to the atmosphere.

Dimethyl sulphide (DMS, $(Me)_2S$). Lovelock *et al.*[14] have found a mean surface water concentration of DMS in the Atlantic Ocean of 1.2×10^{-11} g cm^{-3}. Dimethyl sulphide was not detectable in the marine atmosphere and has not yet been found in the open air. Taking the atmospheric concentration of DMS to be zero, the concentration difference between the sea and the air is 1.2×10^{-11} g cm^{-3}. Substitution in equation (2) along with $K_{1(\mathrm{DMS})} = 19.2$ cm h^{-1} gives $F_{(\mathrm{DMS})} = 2.0 \times 10^{-6}$ g cm^{-2} yr^{-1} and the total flux of DMS from oceans to atmosphere $= 7.2 \times 10^{12}$ g yr^{-1}.

In order to achieve balance, most budgets for sulphur at the Earth's surface have to invoke a process in which some volatile compound (usually H_2S) is produced over the land surface and/or the oceans. A recent sulphur budget[32] gives a figure of 268×10^6 ton SO_4 yr^{-1} (89×10^{12} g S yr^{-1}) for this flux.

The sea–air flux of dimethyl sulphide calculated above (7.2×10^{12} g yr^{-1}) is equivalent to 3.7×10^{12} g S yr^{-1}. This corresponds to about 4% of the amount needed to balance the sulphur budget. It would seem that if, as suggested by Lovelock *et al.*[14], dimethyl sulphide is the "missing" volatile compound in the sulphur cycle then only a small fraction of it is released over the oceans, and a terrestrial source must be sought for the remainder.

Conclusions

The results of the flux calculations presented here are summarised in Table 2. Obviously the results presented are only as good as the data used in the calculations. There is some uncertainty in choosing mean values for the gas and liquid phase exchange constants. For example it is not at all well known from measurements at the sea surface how k_1 varies with wind speed. Even greater uncertainties arise with the air and water concentrations for some of the gases. Many of these occur at low concentrations and require sophisticated apparatus for their detection. The problem is particularly acute for those gases where the degree of supersaturation is small. As further data become available it will be a relatively simple matter to recalculate the fluxes and to extend the approach to other gases. Although the model developed has been applied in this instance to the air–sea system, it should be possible to use it at any air–water interface in the environment provided the necessary data and field measurements are available.

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Temporal Variation of the Seismic Moment Tensor and the Evidence of Precursive Compression for Two Deep Earthquakes

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For two deep earthquakes, about 80 *s before the abrupt onset of each, the hypocentral region begins to experience compression.*

In elastodynamic terms, an earthquake is caused by the sudden release of stress in the Earth. The difference in stress, before and after the earthquake, is termed the stress drop, and the volume integral of the stress drop, which has the dimensions of (force × distance), is termed the moment, or moment tensor. (In displaying the results of numerical calculations we shall use the sign convention consistent with the definitions of the tension and compression axes commonly used in seismological literature.)

The body forces, equivalent to the stress drop, are said to constitute the source mechanism of the earthquake. The balanced double-couple system of equivalent forces has been almost universally adopted in studies of the source mechanism of earthquakes; papers by Randall[1], Knopoff and Randall[2] and Randall and Knopoff[3] represent notable exceptions. Representation of the stress drop by such a system involves two assumptions: (i) the stress is deviatoric; and (ii) the effective stress drop along one of the principal axes is equal to zero. If an earthquake should have, for example, a partly isotropic moment tensor, then studies of its source mechanism based upon the *a priori* assumption of a system of double couples must fail to reveal the isotropic part. It is clear that a procedure in which the elements of the moment tensor can be determined directly from the seismic data is preferable to one that imposes *a priori* restrictions.

It is also important that each of the elements of the moment tensor be independently determined as a function of time (or frequency). Such a general approach can be helpful in establishing the sequence of events at the source and provide data for testing the applicability of various dynamical source models.

New analysis technique

The general approach to the source mechanism problem has now become practically feasible because of the development of a novel method of processing the Fourier spectra of observed seismograms. The particular procedure applied here depends on the availability of digitised recordings from a large number of well distributed stations.

The theory of excitation of normal modes by point sources and the principle of retrieving the moment tensor from seismic data have been described by Gilbert[4,5]. A finite duration (as opposed to instantaneous stress release) of a seismic event implies that the moment tensor is a complex function of frequency. We shall assume, however, that the source represents a point in space; possible errors due to this assumption are largely avoided by considering in the analysis only those modes with wavelengths that are approximately one order of magnitude greater than the expected source dimensions.

Because the temporal changes in the stress release pattern are our principal interest, we shall determine in this report the moment rate tensor—$\mathbf{m}(t)$, the time derivative of the moment tensor—$\boldsymbol{m}(t)$: $\mathbf{m}(t) = \mathrm{d}(t\boldsymbol{m})/\mathrm{d}t$. In the frequency domain, the relationship between the spectra of the moment rate tensor—$\mathbf{M}(\omega)$, and moment tensor—$\boldsymbol{M}(\omega)$ is: $\mathbf{M}(\omega) = i\omega\boldsymbol{M}(\omega)$. Consequently, the procedure described below can be used with only minor changes to determine the spectrum of the moment tensor.

Gilbert's result can be generalised for a source of finite duration; the amplitude of the ground motion due to a normal mode at a certain point on the surface of the Earth is a linear function of the elements of the moment rate tensor $m_j(t)$,

$$u(t,\theta,\phi) = \cos\omega_0 t \cdot \exp(-\omega_0 t/2Q) * \sum_{j=1}^{6} a_j m_j(t)$$

where the symbol $*$ denotes convolution and where the coefficients a_j depend on coordinates θ and ϕ of the receiver, the location of the source and the assumed model of the Earth; parameters ω_0 and Q represent the eigenfrequency and the quality factor of a particular mode. The dependence of u on ω_0 and Q is eliminated by calculating the Fourier transform of the equation above and integrating the resulting cosine and sine transforms over the entire frequency range. Having made observations at a large number of well distributed stations we can solve a system of normal equations for the components of $M_j(\omega_0)$, the Fourier transform of $m_j(t)$ at ω_0.

In reality, the observed spectra represent the superposition of a great many modes and the method cannot be applied as described above. But the spectral energy of a mode is strongly concentrated about its eigenfrequency, and it is sufficient to perform the integration in the frequency domain over a relatively narrow frequency interval in order to retrieve nearly all of its energy. This still leaves open the possibility of interference of another well excited mode with an eigenfrequency close to that of the currently analysed one. But, if the receiver coverage is sufficiently dense, the effect of the modes with angular order numbers different from that of the mode of interest is largely eliminated from the final system of normal equations. The elimination would be complete if the coverage were continuous, because of the orthogonality of the spherical harmonics which enter into the coefficients a_j. This is the same principle which enabled Mendiguren[6,7] and Dziewonski and Gilbert[8] to isolate spectral peaks of normal modes which could not be recognised in the spectra of individual recordings.

Application of the method

Because of the integration procedure described above, the success of the method depends to a large extent upon the precise knowledge of the eigenfrequency of each normal mode for which we attempt to determine the moment tensor. Our previous observations of normal modes[8-10] and inversion studies[9,11,12] have been very helpful in this respect.

Because a moment rate tensor can be expected to vary rather slowly with frequency, we have chosen in this study to determine an average moment rate tensor for a number of adjacent and equal frequency intervals by taking into account excitation of all modes with eigenfrequencies within a prescribed interval and wavelengths greater than an assumed minimum.

The principal advantage of the method proposed here is that in studying the source mechanism we can utilise the entire information contained in a seismic recording. Consequently, the moment rate tensor is derived from the energy of modes representing a wide range of phase velocities, wavelengths and various ratios of compressional and shear energies[9,10]. All previous attempts to determine the source mechanism from the observed amplitudes of surface waves or free oscillations were confined to the fundamental modes. There are, of course, practical limitations on the frequency range of the proposed analysis. The magnitude of an earthquake combined with the instrumental response of the recording stations determines the lower frequency limit. The present upper frequency limit (about 0.014 Hz) is imposed only by the extent of the existing theoretical calculations of eigenfrequencies and eigenfunctions of normal modes.

We have applied the theory and method described above to the analysis of the stress release process of two deep South American earthquakes: the Colombian earthquake of July 31, 1970 (1708 : 05.4 GMT, 1.5° S, 72.6° W, $h = 650$ km) and the Peru–Bolivia border earthquake of August 15, 1963 (1725 : 27.9 GMT, 13.8° S, 69.3° W, $h = 543$ km).

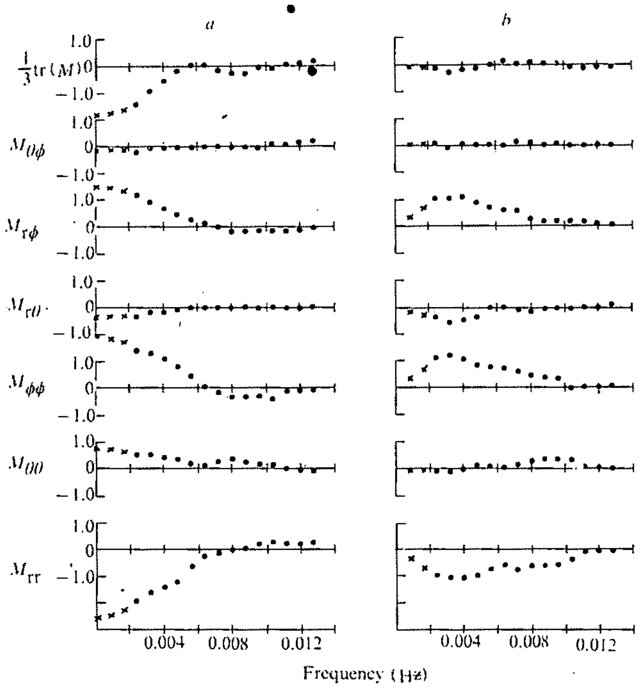

Fig. 1 Moment rate tensor of the Colombian earthquake of July 31, 1970, as a function of frequency. *a*, Real; *b*, imaginary. ●, Values determined from the analysis of excitation of normal modes; ×, extrapolated values. The isotropic component of the moment rate tensor is shown at the top; the remaining diagrams represent the deviatoric part of the moment rate tensor; that is, the isotropic stress rate has been removed for the measured values of the elements M_{rr}, $M_{\theta\theta}$ and $M_{\phi\phi}$. The coordinate system adopted is that of the lower hemisphere with the r axis pointing downwards, θ axis to the north and ϕ axis to the west. To obtain a result in (dyne-cm) the values shown in the figure should be multiplied by a factor of 5×10^{27}.

Figure 1 shows the result of processing 165 recordings of the Colombian earthquake. The centre frequencies of individual intervals were $(1/2\pi)$ .0.005 n Hz ($n = 3, 4, \ldots, 16$) and the half width was $(1/2\pi)$ .0.0025 Hz. The total number of modes considered was 1,348 (only those modes with wavelengths greater than 1,000 km were taken into account) for a total number of observational equations of over 200,000. The number of observational equations for individual frequency intervals was variable but never less than 5,000. Error estimates were obtained from regression analyses but they are too small to be shown in the scale of the figure.

The isotropic stress rate is shown at the top of the figure (negative stress corresponds to compression), and the remaining diagrams represent the elements of the deviatoric part of the moment rate tensor; that is, the isotropic stress rate has been subtracted from the diagonal elements M_{rr}, $M_{\theta\theta}$ and $M_{\phi\phi}$.

If the stress release were instantaneous (a step function in time), the imaginary part of the moment rate tensor should be equal to zero, and the real part should be independent of frequency. This is clearly not the case. If the stress were released uniformly over a period of time from $t = 0$ to $t = t_1$, then the cosine transform should be proportional to sin $\omega t_1/\omega t_1$ and the sine transform to $\sin^2(\omega t_1/2)/(\omega t_1/2)$. With the exception of the isotropic component, this seems to be a relatively good approximation, and the least squares fit yields $t_1 = 78$ s.

The isotropic component shows an entirely different behaviour, with the imaginary component being nearly zero at all frequencies and a significant real component decreasing in frequency more rapidly than all the other elements. This implies a non-zero isotropic stress rate at negative times, or, in other words, a compression preceding the origin time determined from the first arrivals of body waves.

The results of the analysis of 46 digitised recordings of the Peru–Bolivia border earthquake also indicate a release of purely compressional stress at negative times. The elements of the moment-rate tensor for this earthquake are 2.5 times less, on average, than for the Colombian event and the estimate of the uniform radiation time is also lower: $t_1 = 72$ s.

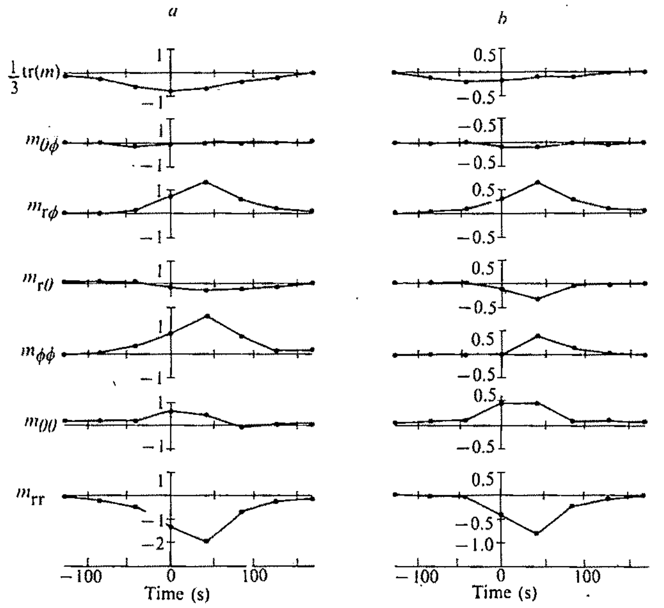

Fig. 2 Moment rate tensors of the Colombian earthquake of July 31, 1970 (*a*) and Peru–Bolivia border earthquake of August 15, 1963 (*b*) as a function of time. Significance of the components of the moment rate tensor is the same as in Fig. 1. To obtain a result in (dyne-cm/sec) the values shown in the figure should be multiplied by a factor of 4×10^{25}.

Our results must be extrapolated for the low frequencies ($n = 0$, 1 and 2) before the frequency domain data can be synthesised into the time domain. Such a procedure is always somewhat arbitrary, but on the basis of theoretical considerations of Aki[13] and the experimental results of Mendiguren[6] for the Colombian earthquake we felt that we were justified in extrapolating the moment rate tensor obtained by the least squares fit of a uniform radiation source. The crosses in Fig. 1 identify values obtained by extrapolation; these values appear to merge smoothly with the experimental data. We believe that the results described later are not materially affected by the uncertainty that might have been introduced by extrapolation.

The time functions of the moment rate tensor are shown in Fig. 2 (it should be remembered that the values shown in the figure do not refer to an instant of time $t = n\Delta t (\Delta t \approx 40$ s) but they represent an average stress release over a period of time from $(n-1)\Delta t$ to $(n+1)\Delta t$). The moment rate tensor decays to a zero value rather rapidly for both positive and negative times, which indicates that the long-period noise is low. The compressional isotropic stress-rate represents a dominating factor at negative times, but there also seems to be a detectable amount of shear stress released during the last 80 s interval before the 'origin time'.

In the next step, we find the principal stress rates and directions of the principal axes of the moment rate tensor for a number of time intervals. The results are shown in Fig. 3, where the axes are numbered according to the decreasing absolute value of the principal stress rate. The directions of the axes are shown only for $t = -40$, 0, 40 and 80 s, because at other times the shear stress rate is comparable to the noise level and the results are incoherent.

The total stress change can be found by integration in time of the results shown in Figs 2 and 3. For example, we have computed the total change in the isotropic stress to be -5.1×10^{27} dyne-cm for the Colombian event and -1.3×10^{27} dyne-cm for the Peru–Bolivia earthquake; using the elastic constants of the model B497[10], we find the decrease of volume to be 1.9 and 0.5 km^3, respectively.

Comparison with other work

Our results are compared with the directions of the compression, tension and null axes found from the fault plane solutions by Mendiguren[6] for the Colombian earthquake and Chandra[14] for the Peru–Bolivia border event.

Our solution is fairly consistent with that of Mendiguren, except for a horizontal rotation of all three axes by approximately 20° and a slow decrease of the plunge of the dominating axis of compression from 79° to 65°.

The differences with the solution of Chandra are much more significant. It seems that his determination of the direction of the tension axis has been in error; it remains at odds with all the other results from this region[15]. Our results show that the azimuth of the tension axis of the average stress released between 0 and 80 s after the origin time is 232°, which is in a good agreement with the trends found from other fault plane solutions by Isacks and Molnar[15]. The most striking result of our analysis of the Peru–Bolivia border earthquake is the significant rotation in time of axes II and III, whereas the position of axis I remains almost unchanged at positive times. This observation indicates that in some cases the pattern of stress release may undergo a significant change during the event.

The validity of a solution for a moment tensor can be confirmed by a theoretical calculation of the excitation of normal modes and comparison of this result with the observed spectra. The method of phase equalising the observed spectra for spectral peaks belonging to individual normal modes represents a severe test of a given source mechanism, since a successful result of this operation depends on the ability to estimate correctly the amplitudes of all the modes within a certain frequency band. We have performed such calculations for the Colombian earthquake data using both the double couple solution of Mendiguren[6] and our results shown in Fig. 1. The results obtained using our solution were generally better than when the double couple model was used. Improvement was especially significant for the modes with a large content of compressional energy and relatively long periods ($T > 200$ s); it can be shown that these modes are particularly sensitive to the isotropic component of a moment tensor. The outcome was also successful when we used our solution for the Peru–Bolivia earthquake, but we have not tested the solution of Chandra. In general, the results of the test confirm the consistency of our moment tensors with the observations.

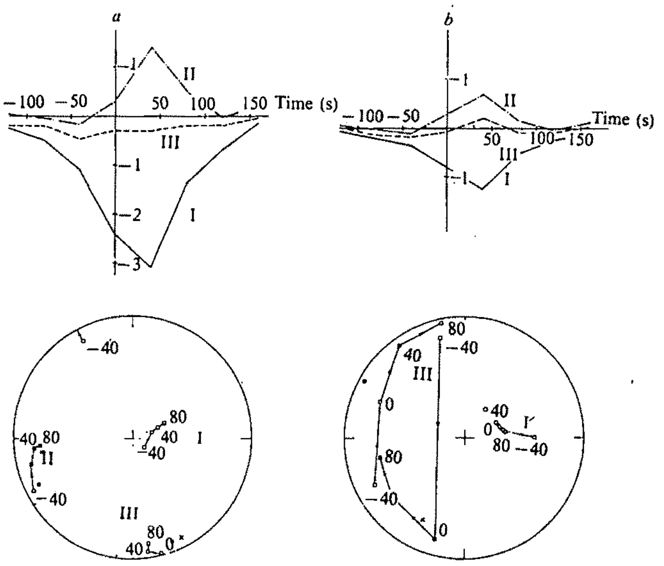

Fig. 3 Temporal variations of the principal stress rates and directions of the principal axes of the moment rate tensor (*a* and *b* and the scale conversion factor as Fig. 2). The large circles in the lower part of the figure represent equal area projections of the lower hemisphere of a focal sphere. Results obtained from fault plane solutions by Mendiguren[3] (left) and Chandra[14] (right) are shown for comparison. ☐, Compression and tension axes of moment rate tensor; ○, ●, ×, compression, tension and null axes from fault plane solutions.

Our results show that a hypocentral compression precedes the main phase of both earthquakes analysed here. This effect may be caused by a transition of the matter in the mantle into a denser phase. If such a change is associated with a release of thermal energy, it might provide an increase in temperature which is necessary to initiate a shear-melting instability which has been suggested by Griggs and Baker[16] as a possible cause of deep earthquakes.

The results in Fig. 3 indicate that some shear stress is also released prior to the 'origin time'. The directions of the principal axes of stress rate at negative times are basically consistent with those determined for the main phases of the earthquakes. The statistical significance of this result is less certain than in the case of isotropic stress, because of the difference of magnitude in the deviatoric part of the moment rate tensor before and after the origin time. But if this result is correct it further confirms the theory of Griggs and Baker which predicts the phase of a relatively slow release of shear stress before the occurrence of shear melting.

Further possibilities

Our results for the two earthquakes seem to confirm that, if the data are adequate, fault-plane solutions provide a reasonably good estimate of the directions and signs of the principal axes of a moment tensor. However, the magnitude of the deviatoric stress rate estimated for axis III represents approximately one-fifth of that of axis I and it is statistically significant. This indicates that although the double couple model may represent a convenient approximation, the mechanism named by Knopoff and Randall[2] a 'linear compensated vector-dipole' could possibly play a significant role in the process of stress release at long periods.

It is not justified to extrapolate conclusions made on the basis of observations in one frequency range into an entirely different frequency range. For example, the temporal variations of the release of isotropic stress indicate that it is a long-period phenomenon; the rate of its release does not appear to change at the 'origin time' (Fig. 2). Consequently, one should not expect to observe a significant effect of a volume change on the amplitudes and signs of the first arrivals of body waves. It would be wrong, of course, to infer from the short period data that a change of volume did not take place, yet such inferences have been made in a number of published papers.

The sequence of events at the source observed for the two analysed earthquakes leads us to the following speculation. If the volume change which preceded the release of the short period energy were insufficient to initiate the shear melting instability, then such a 'silent earthquake' would most likely remain unreported. It may be that hypocentral volume changes which are not followed by the release of short period energy occur frequently. Perhaps the largest of those might have generated free oscillations of the Earth that could be detected by very sensitive instruments.

We believe that analyses such as presented here should be applied to recordings of other events from different tectonic regions and different depth ranges. Information on the stress release prior to the main phase of earthquakes may provide important clues with respect to the problem of earthquake prediction.

Our method of studying the source mechanism can be extended to travelling waves and used to study the source properties of smaller events. In particular, determination of the ratio of energies released by the isotropic and deviatoric part of the moment tensor may be useful as a discriminant between natural events and explosions[17].

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Structural Response of Vertebrate Photoreceptor Membranes to Light

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A distinct structural response in disk membranes from frog retinal rod outer segments was found directly proportional to exposure to light. Translocation of rhodopsin from the intradisk hydrophilic surface to the internal hydrophobic phase of the membrane was observed.

ABSORPTION spectral changes following illumination of the chromolipoprotein rhodopsin in the stack of laminar disk membranes of vertebrate rod photoreceptor cells have generally been interpreted as conformational changes in the lipoprotein moiety[1–4]. Although optical rotatory dispersion and circular dichroism studies of rhodopsin do not seem to support this idea[5,6], it is difficult to arrive at any other molecular interpretation, particularly in view of the established *p*H changes and the exposure of titratable thiol groups that accompany certain of these intermediate processes[3,4]. The question naturally arises as to whether such suspected molecular changes are reflected on the gross structural level of the membrane itself. A substantial amount of rhodopsin[7] in the photoreceptor disk membrane of the frog coupled with the evidence of light-induced conformational changes in the rhodopsin moiety[1–4] indicate that a membrane structural response to light may occur.

Studies of orientated hydrated pellets of isolated disk membranes by X-ray diffraction[8] indicate that structural changes in the disk membranes do occur upon illumination and a slight movement of rhodopsin in the disk membrane upon light exposure has been detected by Blaisie[9,10]. Furthermore, studies of the thermal broadening of X-ray diffraction maxima and the lipid and fatty acid composition of the membrane[11,12] indicate a fluid-like milieu, which makes possible mobility for the chromophore in the disk photoreceptor membrane. In the light of these indications we have attempted to visualise, in a direct manner, such structural changes that might occur in the photoreceptor membrane upon light exposure.

Here we describe the results of our application of freeze-cleave-etch electron microscopy to study photodynamic responses in intact retina, rod outer segments, and disk membranes of the frog, *Rana catesbiana*. The characteristically weak intermolecular interactions in the hydrophobic portion of the disk membrane and the relatively strong interaction of hydrophilic protein with surrounding cell stroma enable this technique to discriminate among three membrane surfaces: the external hydrophilic surface of the disk, the interior hydrophobic region of the disk bilayer, and the internal hydrophilic disk surface, enclosing an internal aqueous region. By electron microscopy of cleaved, shadowed and etched samples, a distribution of these three surfaces can be seen.

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Several preparations of the retina from dark adapted frogs were examined in isotonic Boyle-Conway medium[13], as well as in hypertonic and hypotonic solution. Intact rods were prepared by dissection of whole retina into the Boyle-Conway medium and either flotation of outer segments in 50% buffered sucrose or agitation of the retina and filtration through nylon netting. In addition, pure rods were prepared by density gradient centrifugation and examined. Whole retinae were gently excised and frozen in Freon 22 on stainless steel planchets; these were immediately subjected to a high vacuum (2×10^{-6} torr) at −196° C, in a Denton Co. freeze-etch apparatus. Additional samples were frozen either in the dark or after various stages of light exposure up to 2 min at 25° C. The apparatus containing the planchets was evacuated, the samples cleaved at −197° C and subsequently etched at 95° C for 2 min. The etched samples were then replicated with platinum-carbon, floated on water and taken up on carbon coated copper grids. All electron microscopy was performed with a Hitachi 11-UA instrument.

Membrane surfaces

By this technique we have made many observations on the photoreceptor disk membranes of the frog and have found large differences between dark and illuminated membrane surfaces. In the dark (Fig. 1), all cleaved disk membranes examined showed a smooth outer hydrophilic surface, a particle-free rippled hydrophobic surface and a highly particulated intradisk hydrophilic surface. Identification of these surfaces was made on several bases. First, samples were subjected to etching without previous cleaving. By this technique, it could be assumed that exclusively external disk surfaces were observed. Second, membranes were subjected to light sonication for 10 to 15 s and subsequently etched without previous cleavage. In dark-adapted sonicated samples, only a smooth and particulated surface was observed. The surface bearing particles was similar to the internal hydrophilic surface observed by the cleave-etch technique with dark-adapted membranes. Upon similar sonication treatment of light-adapted samples, only smooth surfaces were observed, implying that translocation of the particles away from the intradisk hydrophilic surface had occurred. Third, a morphologically different surface was evident upon cleavage of the sonicated preparation prior to etching. This surface was the preferred cleavage plane and exhibited a 'rippled' texture. We believe this surface to be the hydrophobic region of the membrane.

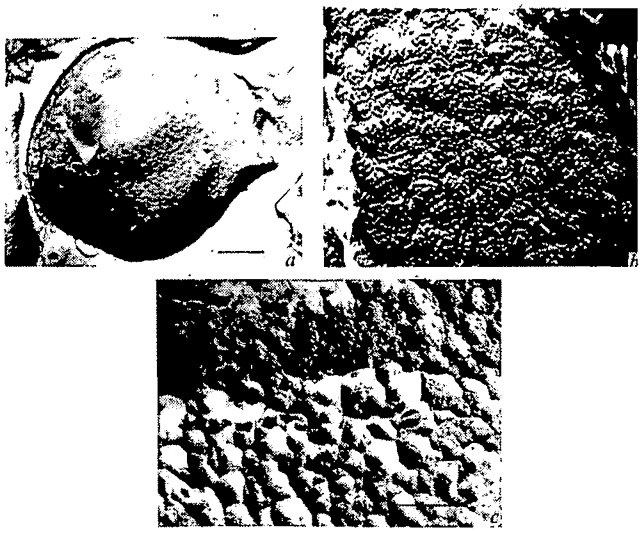

Fig. 1 *a*, Partial surface of dark-adapted photoreceptor disk membrane from preparation of isolated *Rana catesbeiana* rod outer segments; note particle-free outer hydrophilic and rippled hydrophobic surface (bar=0.8 μm). *b*, Partial surface of dark-adapted photoreceptor disk membranes from preparation of isolated *R. catesbeiana* rods; note linear arrays of $\sim$50 to 60 Å particles on intradisk hydrophilic surface (bar=0.18 μm). *c*, Dark-adapted photoreceptor disk membrane fragment from preparation of *R. catesbeiana* rods; note particle-free, rippled hydrophobic region in lower half of figure, and particulated surface immediately above. A smooth membrane area representing an external hydrophilic surface is seen on the upper left hand portion of the figure (bar=0.10 μm). Arrows indicate direction of platinum shadow.

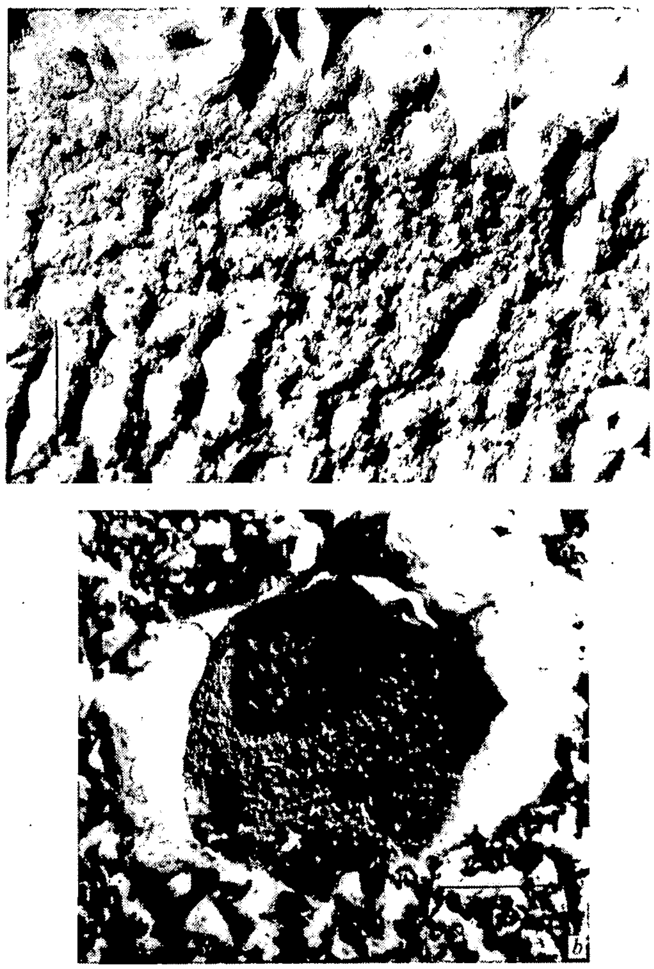

Fig. 2 *a*, Fully bleached disk membrane fragment from preparation of isolated *R. catesbeiana* rods; note particulated hydrophobic surface with $\sim$120–150 Å particles (bar=0.32 μm). *b*, Fully bleached disk membrane fragment from *R. catesbeiana* rods; note particulated hydrophobic surface and adjacent smooth hydrophilic surface (bar=0.12 μm). Arrows indicate direction of platinum shadow.

The particles on the internal hydrophilic surface of the dark-adapted preparations, tentatively identified as rhodopsin, were of the order of 50 Å diameter and seemed to project more than 20 Å beyond the internal hydrophilic membrane surface, although it was difficult to determine the exact height of particles by shadow length in freeze-etched preparations because of random sample tilt in the plane of the incident shadow. Further examination showed the particles to be discrete with a distinct, short-term order relative to other particles, that is, protuberances were arranged in a curvilinear array of ten to twenty discrete particles as seen in Fig. 1*b*. This ordering is not inconsistent with the planar square array reported by Blasie *et al.* on the basis of an optical transform of electron micrographs of isolated disk photoreceptors[14].

After light exposure at room temperature for 2 min at a distance of 4 foot from a standard 150 W bulb to yield predominantly metarhodopsin$_{380}$II in the disk membrane (by spectral monitoring) and quick freezing of the samples, a substantial change in the macroscopic surface structure of the disk membrane was observed (Fig. 2). Once again, a smooth outer hydrophilic surface was apparent. This surface was clearly identified in uncleaved etched samples of disk membranes and showed no change on light exposure. The internal hydrophilic region, however, was now smooth and showed no particulate structure while the inner rippled hydrophobic phase was observed to have a high density of particles of about 125 to 175 Å in diameter projecting 20 to 30 Å above the cleavage surface. In contrast to those seen on the interior hydrophilic surface before illumination, these particles showed no long or short term order and were randomly distributed on the membrane surface. Furthermore, the particles protruding from the hydrophobic surface after light exposure were arranged in what appeared to be aggregates of four to eight molecules and the average distance between centres in the hydrophobic region was smaller than that between particles arrayed on the internal hydrophilic surface of dark-adapted disk membranes.

Rhodopsin molecules

Identification of the observed particles as rhodopsin molecules is based on several points of evidence. The first is the very high density of particles on the membrane surface before and after light exposure, that is, one molecule per 5,400 Å^2. Other known molecular species would not be expected to occur in the disk photoreceptor at this membrane density. The particle density obtained is consistent with that calculated indirectly by X-ray diffraction[15] and microspectrophotometric studies on rhodopsin-containing disk membranes[16].

A second point of evidence is based on observations of membranes from which rhodopsin is extracted by detergent. Lightly sonicated (15 s with a Branson model JI7A sonicator, tuned to 20 kHz, yield of 250 W (acoustical)) disk membranes were extracted in the dark with 3×10^{-4} M aqueous digitonin for 30 min and then centrifuged. The absorbances at 500 nm of the resulting supernatant were taken and the pellet studied by freeze-cleave-etch electron microscopy. It was found that the amount of rhodopsin extracted increases from an initial ΔA_{500} of 0.004 for the first extraction to 0.467 for the third extraction. Electron micrographs of a pellet frozen in the dark after the first extraction revealed the same particle density on the internal hydrophilic disk membrane as untreated membranes with some slight membrane disruption. After the second extraction the number of molecules on the inner hydrophilic surface had markedly decreased to a particle density of one molecule per 14,000 Å^2. The third digitonin treatment disrupts the membrane appreciably, but there is a noticeable decrease in the particle density although it is difficult to quantify.

A third point of evidence derives from the fact that when membranes are fixed in 2% buffered glutaraldehyde in the dark, subsequent light exposure does not induce translocation of the particles to the hydrophobic phase, and the same 50 Å particles remain in linear arrays on the intradisk hydrophilic surface. This observation indicates that the integrity of the lipoprotein must be maintained if the translocation mechanism is to take place. These three points of evidence, though indirect, clearly suggest that the particles seen on the inner membrane are rhodopsin molecules.

On the basis of the observations reported here the native disk protoreceptor membrane seems to be a highly asymmetric protein-lipid bilayer with rhodopsin bound only on the internal disk membrane surface. When the outer hydrophilic membrane surface is visualised with the present technique, it appears, in many cases, to reflect the rippled texture of the hydrophobic phase beneath. On the other hand, no rippling is observed on the inner hydrophilic membrane surface after light adaptation, indicating that this surface is considerably more dense than the outer. These observations are in harmony with the X-ray diffraction studies of Worthington[15] which also suggest an asymmetry of electron density in the disk membranes.

In addition to the above studies, whole intact retina was also subjected to similar treatment. Retinae were studied as fully dark- or light-adapted tissue and in all cases exhibited behaviour identical to the isolated rods and disk membranes. Furthermore, rod outer segments subjected to hypertonic or hypotonic buffer treatment showed respectively shrinking or swelling of the intradisk space, but no deviation in the translocation behaviour within the membrane was observed as a result of this treatment.

Particle translocation

The degree of particle (rhodopsin) translocation is highly dependent on the extent of photobleaching (Fig. 2). Preparations of frog rod photoreceptors were photolysed for 0, 10, 30, 40, 80, 90 and 120 s and samples taken at each bleaching level for freeze-etch electron microscopy. Samples were also taken at each level of bleaching for extraction with 1% Emulphogene in 0.1 M hydroxylamine at *p*H 7.2 and a ΔA_{500} measurement made upon photobleaching of the extracted material. It was observed that the number of particles which were translocated from the internal hydrophilic disk surface to the hydrophobic region of the disk was directly proportional to the extent of photobleaching as measured by spectral ratio. This is further evidence that the particles that translocate in the disk membrane are indeed rhodopsin. In partially bleached samples, the A_{500} absorbance difference at 10 s illumination was 0.59 and at 90 s was 0.018, with the particle density on the hydrophobic surface changing from 15 per 25×10^6 Å^2 at 10 s to 125 per 25×10^6 Å^2 at 90 s (Table 1). When full bleaching occurred, a particle density on the hydrophilic surface was not wholly detectable or possible to quantify. Concomitant with this result, the ΔA_{500} was nearly zero.

Table 1 Absorbance and Particle Density Differences of Frog Rod Photoreceptors Differentially Bleached

Bleaching time (s)*	ΔA_{500}	Particle density† per 25×10^6 Å^2
0	0.068	5
10	0.059	15
30	0.045	35
40	0.042	50
80	0.028	90
90	0.018	125
120	0.008	135

* Exposed at 25° C at a distance of 4 foot from a standard 150 W light bulb with constant agitation of the sample.

† No. on median hydrophobic surface.

The experimental results described above are consistent with the view that the rhodopsin molecules are located on the internal, intradisk hydrophilic surface of the disk membrane. Illumination in conditions terminal at metarhodopsin II seems to initiate a translocation of the rhodopsin molecules to the interior hydrophobic region of the membranes with their subsequent aggregation. This apparent translocation is consistent with a change in the lipoprotein configuration of rhodopsin, manifest as a change in surface properties from a charged hydrophilic to an uncharged hydrophobic character. Such a process, clearly, could quite plausibly be associated with release of a transmitter substance such as calcium ion from the membrane. We stress, however, that although the evidence supporting the location of the rhodopsin molecules on the internal hydrophilic surface of the disk-membrane and their translocation to the median hydrophobic region on illumination is highly indicative, it is not at this stage unequivocal. Furthermore, it should be pointed out that the light levels employed in this study are far from physiological. Normally one might expect at most a few molecules in a given membrane to be bleached within the refractory period of the rod; whether the changes would be qualitatively the same in these conditions as under the conditions of the experiment is a moot point.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Volcanic Couples and Deep Mantle Plumes

MORGAN[1] has convincingly demonstrated a case for the upward rise of deep mantle material along narrow plumes which give rise to positive gravity anomalies and hot spots on the Earth's surface. These hot spots are reflected in large volcanoes and, as Wilson[2] suggested, where the lithosphere moves over hot spots, volcanic chains are produced. Perhaps the most striking support of Morgan's hypothesis are the parallel 'dog legs' in hot spot trajectories given by the Hawaii-Emperor, Austral-Marshall/Gilbert, and Tuamotu-Line chains, most readily explicable as generated by motion of the Pacific plate over three fixed hot spots.

Abundant support for the mantle plume hypothesis is becoming available from a number of approaches[3-6]. Gass[3], Burke and Wilson[4], and LeBas[6] have emphasised that African rifts can be explained by propagation of cracks produced by doming of the African craton, domes which they interpreted as resulting from upwelling mantle plumes. Schilling[7] has explained trace element and isotope variations of Iceland lavas on the basis of mantle plumes. Although this interpretation was criticised by O'Hara[8], independent geophysical evidence[9] also supports the concept of an Iceland mantle plume.

Although much geological, geophysical and geochemical evidence thus seems to be in favour of the hypothesis of deep mantle plumes, the ghost of Kelvin still walks among geophysicists and they have recently issued a statement of their disbelief[10]. Perhaps their disbelief is merely founded in semantics, and could be modified by an answer to Tozer's[11] 'cri de coeur' that someone "explain in terms that are meaningful to geophysicists in what respects the conventional geological pictures of rising magma differ from 'a thermal plume'." Although it is difficult to know what is meaningful to a geophysicist, the following observations on magma production may add some fuel to fire the geothermal plume debate.

Many large central volcanic edifices, both continental and oceanic, are formed from two (and rarely three or even four) volcanic centres spaced about 5 to 25 km apart. These I term 'volcanic couples', and several examples are given in Table 1. Note that many of these are shown as hot spots by Morgan[1].

Table 1 Comparison of Magma Types and Distances Separating Volcanic Centres of Particular Volcanic Edifices

Volcanic edifice (and reference)	Centre 1 basic magma type	Centre 2 basic magma type	Distance (km) between 1 and 2
Hawaii[12]	Kilauea Tholeiite	Mauna Loa Tholeiite	30
K, Ba and so on enriched relative to M. Loa tholeiite			
Réunion[13]	Piton des Neiges Olivine basalt	Piton de la Fournaise Olivine basalt	30
Tahiti[19]	Tahiti-Nui Ankaramite-basanite	Tairapu Basanite-ankaramite	30
Grande Comore[14]	Kartala Alkali basalt	La Grille Basanite	30
St Helena[21]	North-east volcano Alkali basalt	South-west volcano Alkali basalt	~10
Prince Edward Islands[13]	Marion Island Alkali basalt	Prince Edward Island Alkali basalt	30
Ba and K enriched relative to PEI alkali basalts			

An important point about the magmas of these volcanic couples is that some of the centres show fundamental petrological differences which cannot arise by low pressure petrological processes. These differences are mainly related to degree of silica saturation or enrichment in large cation trace elements, for example, Kilauea and Mauna Loa[12], Marion and Prince Edward Islands[13], alkali basalt-basanite (Grande Comore)[14], differences which are now generally interpreted as arising from high pressure processes such as eclogite[15] or orthopyroxene[16] fractionation or degree of partial melting[17]. This point is emphasised by the following discussion of two well documented examples:

The island of Grande Comore lies at the north-western end of a volcanic chain showing classic geomorphological features of age migration, with the oldest in the south-east and youngest in the north-west[18]. (I note that if these volcanoes result from plate movement over a hot spot, this motion was from north-west to south-east, about ninety degrees to the motion suggested by Morgan[1] but more or less parallel to that of Burke *et al.*[5].)

Grande Comore is formed by the coalescence of two volcanoes, Kartala in the south-east and La Grille about 30 km to the north-west. Kartala consists of an alkali basalt suite which shows coherent chemical variations caused by low pressure fractionation of olivine, augite and plagioclase. La Grille consists of a coherent suite of basanites, rich in high pressure ultramafic inclusions, which shows little or no effects of low pressure fractionation. There is a sharp field division between the two volcanoes, and little overlap in chemistry, suggesting independent origins with no mixing of the magmas during ascent. The magmas were interpreted to have arisen from different degrees of partial melting at depths below 60 km, with rapid rise to the surface along conduits definitely less than 15 km in diameter (halfway between the two craters) and probably much smaller[14].

The island of Hawaii consists of five separate volcanic centres separated from each other by distances around 30 km. Like Grande Comore it lies at the youngest end of a volcanic chain which serves as a kingpin in Morgan's mantle plume hypothesis[1]. The following discussion is concerned with only Kilauea and Mauna Loa, although in principle it applies to Mauna Kea, Hualalai and Kohala as well.

Both Kilauea and Mauna Loa have tholeiitic magmas, although the latter are depleted in K_2O and other large cation elements[12]. Wright[12] recognised two kinds of chemical variation in Kilauea lavas, short term variations between each summit eruption caused by high pressure fractionation of hypersthene from magma produced at depths below 60 km and long term variation resulting from a multistage process of fractionation of some combination of olivine, Cr-spinel, orthopyroxene and clinopyroxene, likewise at high pressures. He further suggests that differences between the 1969 Kilauea lavas and Mauna Loa lavas could be explained by the same high pressure fractionation processes which cause differences within Kilauea lavas. Although I accept Wright's interpretation as a reasonable possibility, it should be pointed out that precisely similar differences would result from differing degrees of partial melting.

Irrespective of the details of high pressure magmatic processes responsible, be they partial melting or crystal fractionation, many of the volcanic edifices above hot spots appear to be formed from two (or more) volcanic centres with fundamental differences in magma chemistry which result from high pressure processes. These magma batches may mix, as for Kilauea and Mauna Loa[12], or Tahaiti-Nui and Tairapu[19], or they may separate as in Grande Comore[14]. They generally originate at depths below 60 km and ultramafic inclusions in many indicate rapid ascent to the surface through narrow conduits. If Press's model[22] of oceanic upper mantle is valid and the low velocity zone is at an average depth of 150 km, and if Morgan[1] is correct in his suggestion that the lithosphere is 'de-coupled' at the low velocity zone, then it must be assumed that individual volcanoes cannot be directly related to mantle plumes as a source of magma, except in the special case where the volcano remained stationary above the plume, as suggested by Schilling[7] for Iceland. If the plume itself were contributing material to the volcanoes one would not expect such subtle differences to persist in such precise spatial relationships. Since Iceland-type magmatic differences are found in areas which were not stationary and therefore not fed directly by the mantle plume, one must agree with O'Hara[8] that a direct mantle plume source is unnecessary to explain the Iceland magmas. Further, these relationships suggest that the magmas did not rise directly from the low velocity zone which would presumably be a zone of homogenisation.

Given that deep mantle plumes are not necessarily direct sources of magma, there is less reason to assume that plumes are the result of upward mass transport, that is, of mantle convection. The only necessity for the observed linear patterns of magma generation is a concentrated heat source at the base of the lithosphere—a 'hot spot' or 'thermal plume'. These distinctions between mantle plumes and magma generation may satisfy Tozer's request[11] for terminological clarification, although it is clearly up to the geophysicists to answer some of the resulting questions. For example, do the gravity anomalies which Morgan[1] explained as resulting from mantle plumes have any equally reasonable alternative explanations? Is it possible to have thermal plumes without mass transport, perhaps by zones of high thermal conductivity or concentrated diffusion?

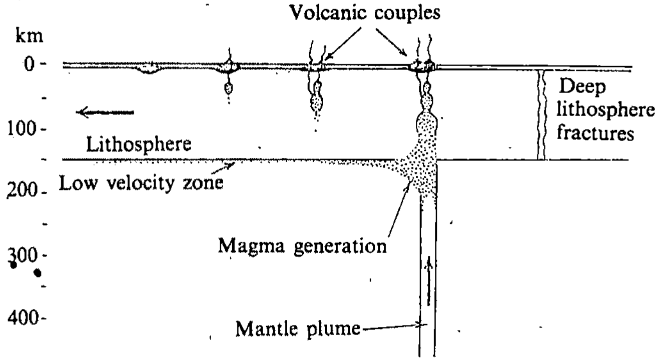

Fig. 1 Magma generated above the mantle plume (either thermal or convective) in or below the low velocity zone is collected in magma chambers and migrates towards the surface after fractionation at a number of intermediate staging areas. Magmas tapped along deep fractures from different staging areas form adjacent volcanoes of different compositions. With migration (to the left) of lithosphere plate above the plume, the magma source is smeared out, the supply of magma is cut off and volcanism is restricted to progressively more extreme fractionation products and eventually terminated entirely, and a new volcanic edifice is formed as other lithosphere 'fractures' (or at least weaknesses) pass over the plume. (Modified from Wilson[2] and Wright[12].)

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Lyoluminescent tissue equivalent radiation desimeter

WHEN some irradiated solids are dissolved in water or certain other solvents, a light emission takes place which, following Wiedemann[1], we call "lyoluminescence." With inorganic materials such as alkali halides, the reactions of trapped electrons from F centres are responsible for the light emission[2]. In the case of some organic materials such as saccharides, trapped free radicals are involved in the process of lyoluminescence[3,4].

It is possible to measure doses between 1 and 10^7 rad with an accuracy of 5% using NaCl and water as a solvent[5]. The stored lyoluminescent energy in NaCl decreases only by 15% after 7 month of storage but it is sensitive to optical and thermal bleaching. Furthermore, the effective atomic number of NaCl is approximately 16, differing considerably from that of human tissue (about 7.5).

The investigation of monosaccharides including glucose, xylose and mannose has demonstrated the stability of the trapped free radials. There was no decrease in their lyoluminescence over 7 month. Their performance has been evaluated with respect to the sensitivity to radiation and stability against thermal annealing. They show linear dependence with doses up to 100 krad. The lower dose detected by the apparatus used (Fig. 1) was 100 rad (ref. 6).

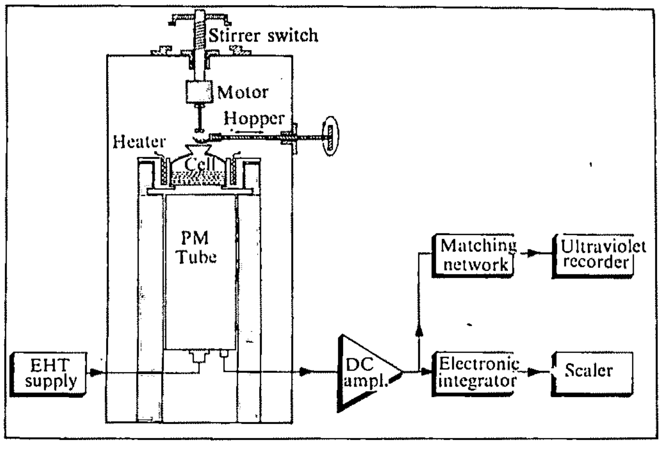

FIG. 1 The lyoluminescence reader

From the shape of the emitted light decay curve, it is evident that there are two or more diffusion controlled reactions involved which differ in their time scale. Here we deal with the short lived component (integrated for 3 s) because it has a much greater intensity than the long lived one.

With NaCl, when using TlCl or fluorescein solution instead of water, the enhancement of light takes place but only at higher doses. With saccharides these fluorescent systems have no effect.

It would be possible to use the lyoluminescence of saccharades for clinical dosimetry if their sensitivity could be enhanced. Therefore the chemiluminescent reactions of luminol (3-aminophthalhydrazide) with oxidising agents was utilised[7,8]. Using a ^{60}Co-γ-source, irradiated saccharides give a bright blue light (≈ 4,460 A°) when they are dissolved in luminol solution. The light enhancement compared to that of water is about 10^6 times. The best results were obtained with trehalose. $2H_2O$ (Fig. 2). It shows an approximately linear light output between 1 and 60^6 rad, and the lower limit is probably controlled by the level of impurities in the system, as luminol is sensitive to metallic ions such as Cu^{2+} and Co^{2+} in higher oxidation states. The slope of the curve is dependent upon the concentation of luminol and the pH of the solution.

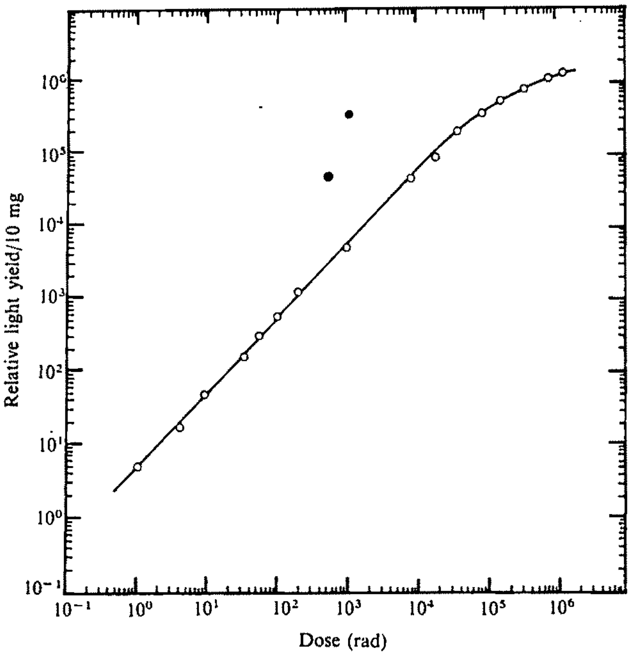

FIG. 2 Integrated light output versus radiation dose, when trehalose. $2H_2O$ was dissolved in 5 ml of luminol solution (125 mg luminol, 1.25 g Na_2CO_3 and 2.5 mg chlorohemine in 1 l deionised water).

It is quite likely that the oxidising species responsible for exciting luminol are formed as a result of free radical reactions with dissolved or adsorbed oxygen in the system. For example, (ROO) is capable of oxidising luminol which is accompanied by the emission of light. Oxidising species were, however, reported to be formed when irradiated saccharides are dissolved in water, and the possibility of H_2O_2 or ROOR formation is not ruled out[9,10]. Thus trehalose. $2H_2O$ which is a fairly true tissue equivalent material makes a good candidate for lyoluminescence dosimetry.

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Newfoundland Ophiolites and the Geology of the Oceanic Layer

THE significance and origin of ophiolites is a matter of interest to geologists. Many investigators interpret ophiolites as slices of oceanic crust which have been tectonically emplaced in orogenic belts[1-7]. Moores and Vine[5], Dewey and Bird[6], and Coleman[7] have proposed just such an origin for ophiolites as geographically diverse as Cyprus, Newfoundland and the Western United States. If ophiolites are representative of the geology of the oceanic crust, then they provide direct access to samples of oceanic crust and the underlying upper mantle—rocks at present inaccessible except for the fortuitous catch in dredge hauls located on tectonic escarpments of the ocean floor. Although we accepted the proposition that ophiolites, if well preserved and not altered by events unrelated to lithosphere accretion, are representative of oceanic crust, we wished first to test this proposition by observing whether representative ophiolite samples had measured velocities compatible with the seismic layering defined for oceanic crust. If the velocities of ophiolite samples were compatible with the range of seismic velocities recorded for oceanic crust, then we hoped to use the ophiolite measurements to understand better the details of the seismic layering of oceanic crust. Here we summarise the acoustic velocity data obtained from metadolerites, gabbros and cumulate gabbros from the Mings Bight ophiolite assemblage[6] located along the northern coast of Newfoundland. A more detailed discussion of these ophiolite samples (J. J. P., in preparation) will give a thorough presentation of petrography, sample density and measured compressional wave velocity of each sample at pressure intervals from 1 atm to 7 kbar.

Seismic models of the oceanic crust have been formulated principally from the interpretation of seismic reflection and refraction data. The velocity structure of the oceanic crust consists of three layers[8]. The oceanic layer (layer 3) is characterised by velocities in the range 6.7 to 6.9 km s^{-1}. Although most reported velocities for the oceanic layer are in this range, the full range of reported velocities is 6.1 to 7.0 km s^{-1}. Overlying this is oceanic basement (layer 2), which exhibits a broad range of velocities (3.5 to 6.0 km s^{-1}), most of which lie in the range 4.4 to 5.6 km s^{-1}. The uppermost layer (layer 1) consists mainly of consolidated and/or unconsolidated sediments having velocities of 1.5 to 3.5 km s^{-1}. Recent seismic refraction studies, however, indicate that the velocity structure of the oceanic crust is more complex than the early studies suggested. Sonobuoy refraction studies of the Reykjanes Ridge show that oceanic basement (layer 2) is made up of two velocity layers[9]. The upper portion (layer 2A) has a recorded velocity range of 2.33 to 3.84 km s^{-1}. The lower portion (layer 2B) is characterised by a velocity range of 4.05 to 5.92 km s^{-1}. Sonobuoy refraction studies in the Pacific show that the oceanic crust (layer 3) is made up of two velocity layers[10,11]. The upper layer is 2 to 3 km thick and has a velocity range of 6.5 to 6.8 km s^{-1} and overlies a lower layer 2 to 5 km thick with a recorded velocity range of 7.0 to 7.7 km s^{-1} (average 7.4 km s^{-1}).

To interpret these seismic models of oceanic crust in terms of lithological units, it is necessary to measure in the laboratory the physical properties of representative samples of rock types recovered from oceanic escarpments. Comparisons of seismic refraction results with laboratory measurements at confining pressures equivalent to *in situ* lithostatic pressures provide information about the possible composition of velocity layers. Several investigators have made laboratory measurements on oceanic samples and have proposed geological models of the oceanic crust. Based on studies of basalts, meta-basalts and meta-gabbros, Christensen[12], Christensen and Shaw[13] and Barret and Aumento[14] proposed that the oceanic crust is composed of basalt and meta-basalt (layer 2) which overlie meta-gabbro (layer 3). We have recently presented the results of a study of basalts, meta-basalts, meta-gabbros, gabbros and serpentinites recovered from the escarpments of the Kane

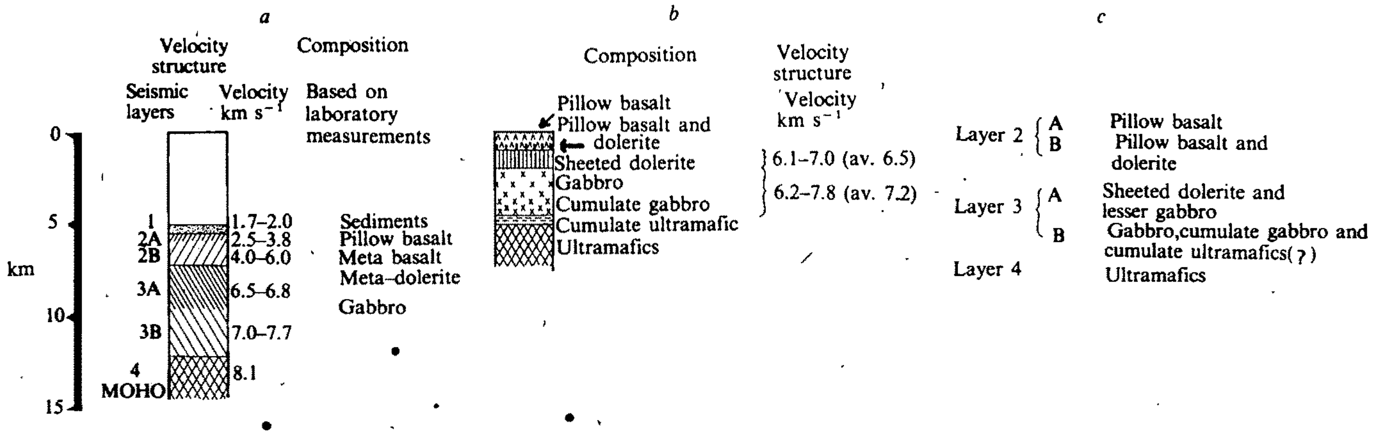

Fig. 1 *a*, Generalised velocity structure of the oceanic crust and the inferred composition of the oceanic crust based on a correlation of velocities measured in the laboratory on dredged rocks with velocities measured by seismic refraction methods. *b*, Generalised geology of Mings Bight ophiolite complex and the measured velocity range of representative dolerite and gabbroic samples. *c*, Correlation between Mings Bight ophiolite assemblage and the layering of the oceanic crust based on laboratory compressional wave velocity measurements.

Fracture Zone[15]. Our results, when integrated with the standard velocity structure of the oceanic crust, led us to propose a slightly different model for the oceanic crust (Fig. 1*a*). We proposed the following structure from top to bottom: layer 2A (2.5 to 3.8 km s^{-1} is made up of a fractured pile of glassy pillow basalts; layer 2B (4.0 to 6.0 km s^{-1}) is made up of zeolite and greenschist facies meta-basalt and minor meta-dolerite; layer 3 (6.7 to 6.9 km s^{-1}) is made up predominantly of unaltered gabbro. The measured velocities of meta-gabbro (greenschist and amphibolite facies), serpentinised peridotite and actinolite-rich rocks were not compatible with the generalised velocity structure of the ocean basins, suggesting that these rock types do not occur in abundance within the oceanic crust but instead reflect tectonic processes associated with the escarpment on which they were sampled. We were intrigued with the seismic refraction results of Maynard[10] and Sutton *et al.*[11] which indicated a high velocity layer ($>$7.0 km s^{-1}) in the lower portion of layer 3. Although only one of the measured gabbro samples had a velocity of more than 7.0 km s^{-1}, we suggested that cumulate gabbros or pyroxenites could be responsible for the high velocity component of layer 3. Rock types of this character had been reported from some fracture zones[16,17].

The ophiolite samples we studied were obtained from the Mings Bight ophiolite complex in north-eastern Newfoundland. These rocks form a steeply dipping zone between the Fleur de Lys metamorphic complex of Cambrian age and Lower Devonian clastics and volcanic rocks. The ophiolite rocks show tectonic contacts and appear to have been tectonically emplaced during the Middle Devonian[18]. This location is ideal because it represents a fully developed ophiolite complex in which all units (see Fig. 1*b*) are represented and in which the rocks have been relatively unaffected by metamorphic episodes associated with the emplacement of the assemblage. Also, as a consequence of locale and climate, ice wedging and wave action cause the altered rock surface to be spalled off and washed away exposing unweathered rock for sampling.

Depending on the sample size, two or three cores, whose axes were oriented so that they were mutually perpendicular, were cut from each sample using a small (0.5 inch) diamond corer. Specimen lengths ranged between 1 and 2.5 inch. The ends of each core were cut smooth and parallel to within 0.001 inch of each other, yielding right circular cylinders. The specimens were jacketed in thin copper foil (2 ml thick) and all joints were sealed with soft solder. The main function of the copper jacket was to prevent entry of pressurising fluid into the pores and cracks of the specimen, so that measurements were made under zero pore pressure, and to serve as electrical shielding, thereby improving the signal-to-noise ratio. To measure the travel time of acoustic energy transmitted through the specimens, a variation of the pulse transmission method of Birch[19] as described by Mattaboni and Schreiber[20] was used. A precision of 1% could be obtained.

The velocity of sound propagated through rocks at low confining pressures depends on the presence of pores and cracks and on their geometry[21,22] resulting in the measurement of markedly lower velocities than would be measured for theoretically dense materials of the same composition. For this reason velocity was measured as a function of hydrostatic pressure. Typically, the greatest effect on velocity occurs within the first 2 kbar of applied pressure. The slope of the velocity curve asymptotically approaches that for theoretically dense material at confining pressures in excess of 2 kbar. The velocities reported here are those determined at 1.5 kbar, which is within the range of *in situ* lithostatic pressure for the oceanic layer (layer 3A, 3B). The density of the specimens was calculated from direct measurements of their lengths, diameters and masses, and is regarded as accurate to within 1%.

The results of thirty-two measurements on thirteen samples of gabbro and cumulate gabbro are shown in Fig. 2. We display the individual measurements, rather than averaged results, to emphasise the spread in the velocity range that might be measured for a given rock type. The leucocratic to melanocratic gabbros commonly exhibit ophitic textures and are composed in general of calcic plagioclase and clinopyroxene with minor vein and interstitial serpentine, fibrous amphibole and chlorite. The plagioclase of the gabbros is often saussuritised. The cumulate gabbros are characterised by bands enriched in pyroxene and olivine within the gabbro. Minor serpentine and fibrous amphibole are associated with the cumulate gabbros. The calculated densities of the gabbro and cumulate gabbro specimens range from 2.9 to 3.2 g cm^{-3}. The compressional wave velocity range of these gabbroic samples at 1.5 kbar confining pressure is 6.1 to 7.8 km s^{-1}. The majority of the samples have measured velocities above 6.9 km s^{-1} with a well defined peak at 7.4 to 7.5 km s^{-1}. The six samples with velocities below 6.9 km s^{-1} exhibit a larger amount of fibrous amphibole and chlorite than the higher velocity samples. We observed in a study of oceanic gabbro that the development of amphibole and chlorite at the expense of primary mineral phases decreases the compressional wave velocity[15].

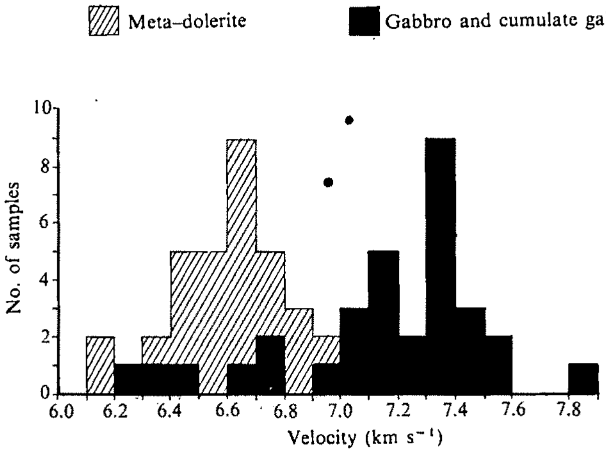

Fig. 2 Histogram of measured velocities for thirteen samples of gabbro and eleven samples of meta-dolerite (greenschist facies) from Mings Bight ophiolite complex. Two or three specimens, oriented so that they were mutually perpendicular, were cut from each sample. The measured velocity for a given specimen displayed individually. The velocity results shown here were measured at a confining pressure of 1.5 kbar which is within the range of *in situ* lithostatic pressure for the oceanic layer (layer 3).

The results of twenty-seven measurements on eleven samples of greenschist facies meta-dolerite are shown in Fig. 2. The meta-dolerites often exhibit relict sub-ophitic textures and are composed of plagioclase, sometimes enriched in Na, and pyroxene with varying amounts of epidote, chlorite, actinolite and quartz. The calculated densities of the meta-dolerite samples vary from 2.8 to 3.0 g cm^{-3}. The compressional wave velocity range of the dolerites at 1.5 kbar confining pressure is 6.1 to 6.9 km s^{-1}. At a confining pressure of 0.75 kbar (calculated *in situ* lithostatic pressure range for layer 2) the velocities of the dolerites range from 6.0 km s^{-1} to 6.9 km s^{-1}, indicating that dolerites of the sheeted dike layer are not representative of seismic layer 2B (velocity range of 4.0 to 6.0 km s^{-1}).

The measured compressional wave velocities of the dolerite and gabbro samples range from 6.1 to 7.8 km s^{-1}. Seismic refraction measurements of layer 3 (A and B) of the oceanic crust show a similar range of velocities[8]. This correlation supports the interpretation that ophiolite assemblages are representative of oceanic crust. The histogram of measured compressional wave velocities (Fig. 2) exhibits a distinct bimodality with a dolerite peak at 6.6 to 6.7 km s^{-1} and a gabbroic peak at 7.4 to 7.5 km s^{-1}. These velocities compare well with the bimodal distribution of seismic velocities recorded for layer 3A (6.5 to 6.8 km s^{-1}) and 3B (7.0 to 7.8 km s^{-1})[10,11]. Accepting the proposition that ophiolites are representative of oceanic crust, we conclude from our data that the upper portion of the oceanic layer (seismic layer 3A) is composed predominantly of sheeted dolerite and possibly some gabbro. The lower portion of the oceanic layer (seismic layer 3B) is composed predominantly of gabbro and cumulate gabbro. Based on velocity alone it is difficult to say conclusively whether the cumulate ultramafic rocks (pyroxenites) belong with layer 3B or upper mantle. We sampled several pyroxenities for this study but all the samples have been serpentinised, resulting in anomalously low velocity. Some published values of unaltered pyroxenites at 1.5 kbar confining pressure indicate that rocks of this type have velocities in the range 7.3 to 7.7 km s^{-1} and therefore could be a constituent of layer 3B. More work is needed to define the velocity range of pyroxenites but, based on the results to date, we propose that the cumulate ultramafics belong with the gabbroic rocks of layer 3B.

Poster[23] has recently reported results on velocity measurements of the Troodos ophiolites. His results for three gabbros are similar to those reported here; two of the three diabase measurements, however, are lower than those reported here, presumably because of greater alteration. Nonetheless, he arrived at a conclusion similar to our model. If the ophiolite assemblages are analogues of the geology of the oceanic crust, then it is clear that the petrological associations within the oceanic basement and oceanic layer are complex. Indirect geophysical measurements of the physical expression of the oceanic layers do not have the resolution to resolve the inherent complexity of these layers and therefore careful study of ophiolite assemblages may hold the key to the details of oceanic lithosphere geology.

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Molecular Complexity of Water Vapour and the Speed of Sound

SEVERAL real atmosphere and laboratory spectroscopic measurements[1-3] suggest that water vapour near saturation contains polymeric molecules of which the concentration rises with increasing pressure and decreasing temperature. Existing *PVT* data which could support this conclusion lack the necessary accuracy for temperatures which are approaching atmospheric values and no related speed of sound measurements seem to be available for the conditions of interest.

We give here a preliminary report of some new measurements derived from observations of the transit time of sound pulses in water vapour. The measurements were made at a fixed temperature and the vapour pressure was varied from low values up to values close to saturation. Results are shown in Fig. 1.

A gas such as neon, which shows no molecular association, gives speed values which are independent of pressure, so the trend we observe in water vapour of speed decreasing with increasing pressure is taken as showing that it contains molecular complexes. The speed decrement with pressure becomes greater as temperature is lowered. To compare with the observations we have considered a model in which the complexes are all taken to be dimers. This model will be represented by straight lines in the plot since the speed of sound varies as the square root of gas density and dimer concentration will vary as its square. The fractional dimer concentration is measured by the slope of these lines and at 50° C and 90% saturation we find a value of 8×10^{-3}. The temperature dependence of the slope is a measure of the dimer binding energy for which a value of 0.5 ± 0.1 eV per dimer molecule is found.

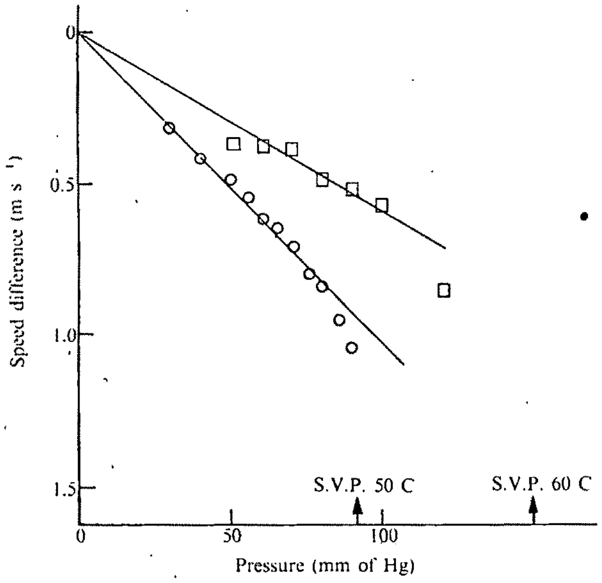

Fig. 1 Speed of sound in water vapour. The ordinate is the difference between the observed speed and the speed, appropriate to the temperature, extrapolated to zero pressure. □, 60° C; ○, 50° C.

The model used for analysis is only a first approximation and is probably oversimplified: the values also are tentative and subject to confirmation when the measurement technique can be refined, but these speed of sound measurements clearly show molecular complexity in water vapour at temperatures approaching tropospheric values.

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Light Sensitivity of Tests for Cadmium on Ceramic Tableware

Many countries, including Ireland, Great Britain and the United States, have introduced national standards aimed at controlling the amount of lead and cadmium which can be leached into food from ceramic tableware[1-3].

Most countries including those mentioned above favour a cold test while others, notably Denmark, favour a boiling test of shorter duration[4]. The cold test usually involves filling the article with 4% acetic acid and allowing it to stand for 24 h. Limits are set for the concentration of lead and cadmium in the resultant leaching solution. In the Irish and United States tests, the limits are set at 7 p.p.m. for lead and 0.5 p.p.m. for cadmium. The British Standard (BS) for ceramic flatware is 20 and 2 p.p.m. for lead and cadmium respectively, those for hollow ware below 1,100 ml are 7 and 0.7 p.p.m. for lead and cadmium respectively and the BS limits for hollow ware of 1,100 ml and above are 2 and 0.2 p.p.m. for lead and cadmium respectively. The method of test is, however, very similar to that used in the American and Irish tests.

The series of experiments reported here were carried out on whiteware pottery with a lead-bearing glaze on which was applied a multicoloured silk-screen onglaze pattern, known to contain cadmium. A series of plates, decorated, heat treated and tested at room temperature in a similar manner in the absence of light, gave values of 0.17 ± 0.06 p.p.m. for cadmium. But, if plates from a similar batch were tested under daylight conditions or under artificial light at similar temperatures, results up to 2.5 p.p.m. could be recorded depending on the duration, intensity and wavelength of the radiation allowed to fall on the plates in the 24 h period. The results also indicate that significant leaching can occur in brightly lit conditions when the 4% acetic acid solution is replaced by deionised water as the leaching solution. Results of 0.5 p.p.m. have been recorded, once again depending on the duration and intensity of the light in the 24 h period.

Throughout the experiments no change was noted in the results for the amount of lead leached, which remained at 1.2 ± 0.4 p.p.m. When distilled water was used, no lead was leached into solution.

There is also evidence which indicates that the boiling test used in Denmark can be similarly light sensitive.

We suggest that, in the case of the series of results reported here, the light sensitivity of the test is due to the photooxidation of insoluble cadmium sulphide to soluble cadmium sulphate. Cadmium sulphide has been shown by X-ray diffraction to be present in the onglaze decoration. Clearly, articles may pass or fail the various national specifications according to the light conditions in which the test is carried out. Further details of the results quoted here will be published elsewhere.

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BIOLOGICAL SCIENCES

Absorption of Cadmium, Copper and Zinc by Dog Whelks in the Bristol Channel

SEVERAL independent studies have established that unusually high concentrations of certain heavy metals, most notably cadmium, are found in littoral flora and fauna of the Bristol Channel[1-4]. An experiment by Peden *et al.*[5] established that the concentrations of cadmium and zinc in dog whelks (*Nucella lapillus*) did not markedly decrease after transferring specimens to clean water for seven weeks. Bristol Channel dog whelks have shells with an elongated shape, clearly distinguishable from the usual shell shape. This feature, together with the fact that dog whelks move very little (J. H. Crothers, personal communication), makes the species ideal for studying the absorption of metals.

In April 1973, 120 dog whelks with the usual shell shape were collected at Beer, in Dorset (National Grid reference SY235895). Ten specimens were analysed for cadmium, copper and zinc, and the rest were released in a clearly identifiable area of approximately 500 m^2 at St Andrew's Head, North Somerset (National Grid reference ST324662). The area was below the *Fucus serratus* zone (below the Mean Low Water Level), where the local dog whelks live, feeding on acorn barnacles (*Elminius modestus* is the dominant species here). Specimens were collected after 1 week and thereafter at monthly intervals. The survival rate was good, although some fatalities were noticed at the end of June, when the spring tides uncovered the animals in early afternoons in hot sunlight. A similar proportion of fatalities was noticed among the local dog whelks. Even after 6 months no specimen was found outside the release area.

After removing the shell, each specimen was weighed, dried at 105° C for 24 h, reweighed and dissolved in 5 cm^3 concentrated nitric acid (AR grade). The solution was diluted to 25 cm^3 with double-distilled water, and the metal concentrations were determined by atomic absorption spectrophotometry (Table 1).

Several common limpets (*Patella* sp.) were collected from Beer at the same time as the dog whelks. The shells were scratched with a cross and released at St Andrew's Head on a rock in the *Fucus serratus* zone. Local limpets from the same spot were collected at the time of transplanting the specimens. The survival rate was poor and the small collections were made at irregular intervals (Table 2).

The concentration of cadmium in the dog whelks increased at an appreciably slower rate than that of zinc. At the end of the fourth month, the cadmium concentration was significantly lower than that in the native specimens, but it was still increasing steadily. By the end of the second month, the zinc concentrations were statistically indistinguishable from those in the native specimens. The copper concentrations remained considerably less than those in the native specimens.

Because of the small number of specimens analysed, only speculative observations about the uptake of metals by limpets

Table 1 Concentrations of Cd, Cu and Zn in *Nucella lapillus* Transferred from Beer (Dorset) to St Andrew's Head (North Somerset)

Sample details	Date	No. of specimens	Cd	Cu	Zn
Beer, before transplant	April 8, 1973	10	36 (11–62)	70 (20–110)	345 (110–480)
	Standard deviation		14	23	95
Transplanted specimens	April 18, 1973	6	40 (19–74)	140 (60–260)	620 (190–1,150)
	Standard deviation		17	74	400
Transplanted specimens	May 6, 1973	10	63 (44–93)	175 (70–370)	1,400 (890–2,350)
	Standard deviation		18	82	450
Transplanted specimens	June 2, 1973	6	92 (62–140)	120 (80–180)	2,000 (1,360–6,450)
	Standard deviation		27	33	2,000
Transplanted specimens	July 1, 1973	6	135 (117–150)	113 (78–150)	2,800 (1,700–3,600)
	Standard deviation		12	29	600
Transplanted specimens	July 29, 1973	7	184 (132–226)	200 (105–330)	3,200 (1,800–4,400)
	Standard deviation		34	78	850
Transplanted specimens	August 27, 1973	6	211 (167–243)	170 (80–390)	2,530 (1,700–4,330)
	Standard deviation		27	100	950
Local specimens	December 1972	10	460 (270–580)	740 (405–1,010)	3,090 (1,300–4,500)
	Standard deviation		105	180	1,100
Local specimens	August 28, 1973	11	780 (500–1,120)	905 (385–1,750)	2,900 (1,460–4,030)
	Standard deviation		200	380	1,050

Mean values (with extreme values in parentheses), p.p.m. in the dry flesh.

Table 2 Concentrations of Cd, Cu and Zn in *Patella* sp. Transferred from Beer (Dorset) to St Andrew's Head (North Somerset)

Sample details	Date	No. of specimens	Cd	Cu	Zn
Beer, before transplant	April 8, 1973	11	11 (3.5–28)	7 (3.5–12)	91 (55–130)
Transplanted specimens	April 15, 1973	4	10 (3.5–17)	11 (8–18)	115 (90–140)
Transplanted specimens	May 6, 1973	3	14 (10–20)	17 (15–22)	185 (170–205)
Transplanted specimens	July 29, 1973	2	200 (100, 300)	30 (28, 33)	235 (235, 235)
Local specimens	December 1972	9	220 (67–440)	30 (ND–50)	340 (290–41)

Mean concentrations (with extreme values in parentheses), p.p.m. in dry flesh. ND, Not detected.

can be made. But it does seem that limpets absorb both copper and cadmium more rapidly than dog whelks.

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Use of Sulphur-coated Urea for Revegetation of Blast Furnace Slag

Apart from studies in the Lower Swansea Valley[1–4] there has been no attempt to investigate the factors inhibiting plant growth on blast furnace slag. This material is probably second only to colliery spoil as a mineral waste problem in the United Kingdom.

Recent investigations on the revegetation of blast furnace slag in Lancashire have shown that growth is limited by high *p*H (10.2 to 10.5) and serious deficiencies of the major nutrients nitrogen and phosphate (my unpublished work). Although exposure of the waste causes a reduction in *p*H at the surface due to leaching of hydroxides, the underlying material remains highly alkaline (Table 1). Consequently, plant growth is restricted by the limited depth of the hydroxide-free rooting substratum. The waste is also highly porous so that soluble nutrients such as nitrogen are quickly lost from the planting medium.

The provision of adequate phosphate for growth establishment is not difficult and some slags may even contain this nutrient. For the reasons described, however, soluble inorganic forms of nitrogen are most inefficient when used on this waste. I have therefore investigated the efficiency of sulphur-coated urea as a slow release nitrogen source in order to achieve a visually acceptable grass cover on the waste without involving the provision of expensive manurial or soil top coverings.

A randomised block experiment was set up on a levelled tip site with a surface *p*H of 9.0 after some 4 months of exposure. Five different nitrogen treatments were investigated, namely, nil N, 50 and 100 kg ha^{-1} N as urea, and 50 and 100 kg ha^{-1} N as sulphur-coated urea. Phosphate was applied to all the plots at 75 kg ha^{-1} P_2O_5 and *Festuca rubra* which is relatively tolerant of the waste conditions[4] was sown in June 1972. In early September 1972 there was evidence of phosphate deficiency. Accordingly, the plots were refertilised with 125 kg ha^{-1} P_2O_5 and three nitrogen treatments were superimposed on the initial treatment regimes according to a split plot design. These were nil N, 100 kg ha^{-1} N as urea, and 100 kg ha^{-1} N as sulphur-coated urea. In this way, the efficiency of slow-release nitrogen was investigated as an initial and maintenance fertiliser treatment.

Table 1 *p*H Profile of Blast Furnace Slag after 12 Months Exposure

Depth (cm)	*p*H
0	8.1
2.5	9.0
5.0	10.2
7.5	10.3
10.0	10.5
15.0	10.4

All the plots were assessed visually by four independent observers in late May 1973. The criterion of assessment was overall visual acceptability as affected by percentage cover of the substratum, colour and uniformity of growth, and sward density. Each of the experimental grass plots was designated an index of acceptability within the range 1 to 10 and the results were assessed by statistical analysis.

Table 2 shows the mean indices of growth acceptability in relation to the initial and secondary nitrogen treatments. It can be seen that overall growth was still influenced by the initial nitrogen applications at this time. The residual slow-release nitrogen responses were significantly better than those obtained by the use of soluble urea nitrogen. It is interesting that the performance from the low rate of slow-release nitrogen was superior to that from the high rate of urea nitrogen. Considering the effects of the second fertiliser treatments, slow-release nitrogen was again significantly better than uncoated urea. Therefore, both initial and maintenance applications of slow-release nitrogen give better growth than soluble nitrogen.

Table 2 Mean Indices of Visual Acceptability as Affected by Nitrogen Fertilisation

Initial N treatments	Nil	N1	N2	SN1	SN2
	3.30	3.83	4.43	4.73	5.37
	l.s.d. at $P=0.05=0.53$				
Second N treatments	Nil		N		SN
	1.54		4.94		6.52
	l.s.d. at $P=0.05=0.41$				

N, Urea nitrogen.
SN, Sulphur-coated urea nitrogen.

Table 3 shows the interactions of the initial and second nitrogen treatments. Clearly, nitrogen is better applied after planting if one fertiliser treatment only is involved. For good growth, however, it is obviously necessary to provide two additions, both of which should be in slow-release form for best results. If soluble and slow-release nitrogen treatments are combined, it is preferable to use the slow-release form at the second fertilisation rather than the first.

Table 3 Mean Indices of Visual Acceptability in Relation to Initial and Maintenance Nitrogen Regimes

		Maintenance N		
		Nil	N	SN
	Nil	1.00	3.80	5.10
Initial N	N	1.00	4.80	6.60
	SN	2.35	5.65	7.15
		l.s.d. at $P=0.05=0.91$		

Indices for initial nitrogen treatments are means of high and low levels of application.

The critical limit of visual acceptability at the time of assessment was represented by an index of about 6.0. Since this was only exceeded following treatment with slow-release nitrogen, it is clear that the choice of slow-release against soluble nitrogenous fertiliser can determine the success or failure of acceptable vegetation establishment on blast furnace slag. These results may have important implications in the revegetation of other industrial waste materials where similar problems of substrate toxicity and adverse nutrient retention are involved.

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N-terminal Acetylation of Histone-like Nascent Peptides on Rat Liver Polyribosomes *in vitro*

Several proteins are known to have the amino terminus blocked by acetyl radicals[1]. At least six of these proteins, including the f_{2a} histones, have an N-acetylserine terminus[2]. The mechanism by which the acetyl group becomes attached to these molecules is not well understood. Proposals suggesting the initiation of protein synthesis by acetylated amino acids as a general mechanism in eukaryotes[3] have been refuted[4]. However, a role of N-acetyl amino acids in the initiation of the synthesis of certain specific proteins cannot be ruled out[5]. An explanation for the presence of N-acetyl amino acids at the amino terminus of proteins is the existence of an acetylating enzyme specific for certain N-terminal sequences in proteins or growing polypeptides chains. The finding of an acetyltransferase activity bound to rat liver ribosomes and specific for the N-terminal amino acid of some proteins associated with the ribosomes[6] offers an appropriate system for the study of the alternative mechanism. We report here the analysis of histone-like nascent peptides acetylated *in vitro* in liver polyribosomes prepared from rats hepatectomised 22 h before being killed. The results indicate that acetyl groups can be attached directly to N-terminal serine residues of nascent peptides in the absence of the initiation of protein synthesis.

The proposed role of N-acetylserine tRNA as an initiator of the synthesis of f_{2a} histones results from the finding of N-acetylserine in growing peptide chains isolated from regenerated rat liver and the finding of N-acetylserine tRNA in the cytoplasmic extracts from the same source[5]. The first of these two observations indicates that acetyl groups are attached before histone synthesis is completed, but it does not necessarily imply an initiation mechanism, since the N-terminal acetylation of polypeptide chains during chain elongation gives the same result. To explore this possibility, polyribosomes prepared from rats partially hepatectomised 22 h earlier were acetylated *in vitro* by incubation with 1-^{14}C-acetyl coenzyme A (ref. 9). Polyribosomes were also acetylated *in vivo* with ^{3}H-sodium acetate[8] under conditions in which N-terminal acetylation of histone nascent peptides is known to occur[5]. Aliquots of polyribosomes acetylated *in vivo* and *in vitro* were analysed by sodium dodecyl sulphate-acrylamide gel electrophoresis to compare the distribution of acetyl radicals among the proteins resolved in this system. As Fig. 1 shows, two groups of acetyl proteins with molecular weights of 30,000 and 10,000–20,000 were present in polyribosomes acetylated *in vivo* and *in vitro*. We also found that most proteins acetylated *in vivo* had a molecular weight greater than 30,000, and no correspondence with the proteins acetylated *in vitro*. The reason for this disparity is not known.

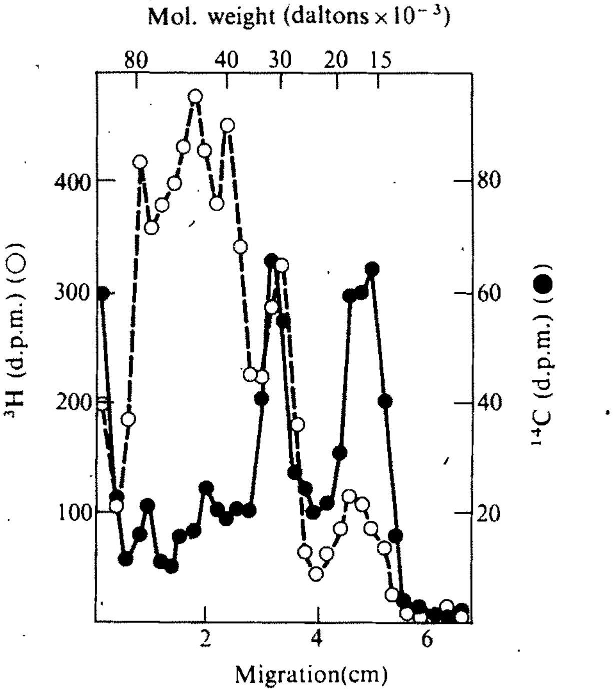

Fig. 1 Sodium dodecyl sulphate acrylamide gel electrophoresis of polyribosomes acetylated *in vivo* and *in vitro*. Liver polyribosomes were prepared[7] from rats 22 h after partial hepatectomy. One group of rats was given 10 mCi per rat of ^{3}H-sodium acetate before death to obtain polyribosomes acetylated *in vivo*[5]. Polyribosomes from untreated rats (250 A_{260} units) were acetylated *in vitro* by incubation with 0.05 mCi of 1-^{14}C-acetyl coenzyme A in the presence of 0.05 M Tris-HCl, *p*H 7.5, 0.1 M KCl and 1 mM dithioerythrol[9]. Aliquots (4–5 A_{260} units) were analysed in 10% acrylamide gels by electrophoresis in the presence of 0.1% sodium dodecyl sulphate and the radioactivity in the gel fractions was counted as described[9]. The solid and open circles correspond to ^{14}C and ^{3}H-radioactivity, respectively.

To study the presence of acetyl radicals in the histone-like nascent peptides, we incubated the polyribosomes with puromycin in the presence of a high concentration of salt and they were then pelleted by high speed centrifugation. The growing peptides released by puromycin were then treated with 0.25 N sulphuric acid to extract the basic proteins and polypeptides, which were then dialysed for 48 h against 0.025 N sulphuric acid, precipitated with 4 volumes of absolute ethanol in the cold and dried under vacuum. (About 2.5 mg of dried product is recovered from 20 g of wet liver.) In terms of radioactivity, the recovery was less than 5% of the total radioactivity found in polyribosomes. The basic protein and growing polypeptides in the acid extract were characterised in two acrylamide gel electrophoretic systems. In sodium dodecyl sulphate electrophoresis (Fig. 2*A*), all the acetyl radioactivity was found associated with proteins having apparent molecular weights of 10,000–20,000; in this range of molecular weights is found histone f_{2a} (ref. 11) or its nascent peptides near completion. Electrophoresis in the presence of urea at *p*H 2.5 (Fig. 2*B*) revealed that most of the acetyl protein in the sample had a mobility close to the histone standards. From this study we conclude that most of the acetyl protein released from polyribosomes by puromycin and extracted by dilute sulphuric acid represents histone nascent peptides near completion; moreover, the results also indicate that histone nascent peptides on polyribosomes can be acetylated *in vitro*.

The acetyl groups in the histone nascent peptides were characterised by a combination of ion exchange filtration and paper electrophoresis and chromatography. The histone nascent peptides from polyribosomes acetylated *in vitro* and *in vivo* were digested with trypsin, pronase and carboxypeptidase[9]. The resulting solution of acetyl amino acids and peptides was passed through a Dowex 50 H^+ column and the non-absorbed material was eluted with water to obtain the 'acidic' fraction, which contains N-terminal blocked amino acids and peptides. All the absorbed material was eluted with 0.5 N ammonium hydroxide to obtain the 'basic' fraction, containing all the free amino acids and peptides. Radioactivity measurement of these fractions indicates (Table 1) that most of the acetyl groups introduced *in vitro* were associated with the 'acidic' fraction and therefore represent N-terminal acetylation. Only 13% of the acetyl groups attached *in vivo* were found in the 'acidic' fraction. We also tried to identify the N-acetyl amino acids in the 'acidic' fractions. Analysis by paper chromatography (Fig. 3*A*) showed that most of the acetylated material has the same R_F as the standard N-acetylserine (10 cm). Analysis in paper electrophoresis (Fig. 3*B*) also shows the same mobility for the standard acetylserine and the acetyl amino acids in the 'acidic' fraction derived from the nascent peptides acetylated *in vitro*.

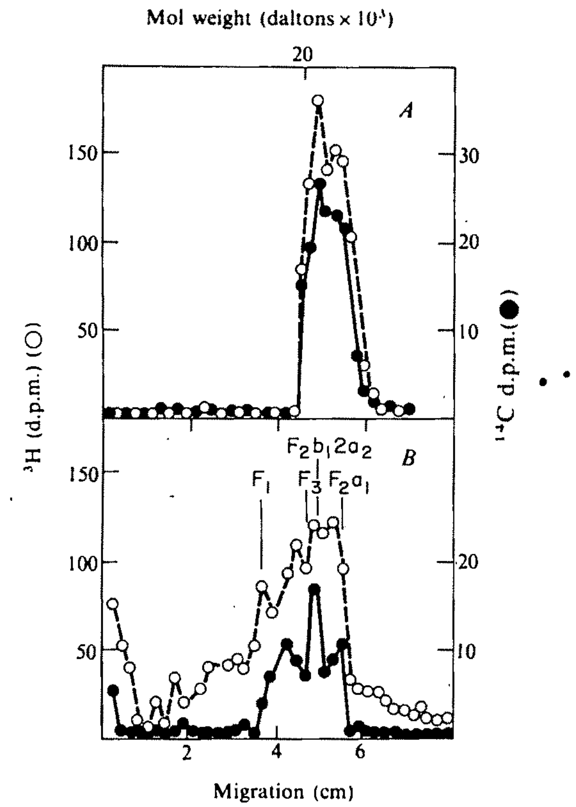

Fig. 2 Electrophoresis of histone nascent peptides extracted from acetylated polyribosomes. Nascent peptides were released with puromycin[4] from polyribosomes acetylated *in vivo* and *in vitro*. Histone nascent peptides were extracted from the puromycin supernatants as described in the text. Samples of 100–200 µg of the dried material containing histone nascent peptides were resuspended in 1 N acetic acid, 10 N urea and 1% mercaptoethanol, and analysed by (*A*) sodium dodecyl sulphate-acrylamide gel electrophoresis as described in Fig. 1, or (*B*) urea-acrylamide gel electrophoresis at *p*H 2.5 (ref. 11). Symbols are as in Fig. 1. Histone standards were also run in parallel and were located by scanning the gels at wavelengths 280 nm, 600 nm, after staining with Coomassie blue[6].

Table 1 Dowex 50 H^+ Fractionation of ^3H-^{14}C-Acetyl Peptides from Histones Extracted from Polysomes of Hepatectomised Rats*

Fraction	^3H d.p.m.	%	^{14}C d.p.m.	%
'Acidic' (N-terminal blocked)	720	13	1,470	96
'Basic' (N-terminal free)	4,470	85	57	5

* Histone nascent peptides (about 1.5 mg) extracted from polyribosomes acetylated *in vivo* and *in vitro* were resuspended in 2 ml of 0.1 M sodium bicarbonate, *p*H 8. Enzymatic digestion with trypsin, pronase and carboxypeptidase A and B was performed as described[9]. After removal of any cold trichloroacetic acid (TCA)-insoluble material by centrifugation and extraction of TCA from the supernatants with ether, the digests were dried under vacuum, resuspended in 2 ml of water and absorbed onto 1 × 8 cm Dowex 50 H^+ columns. The columns were washed with 20 ml of water followed by 20 ml of 0.5 N ammonium hydroxide to obtain 'acidic' and 'basic' fractions respectively. The radioactivity in duplicate 0.2 ml aliquots from the fraction was measured by liquid scintillation counting with 10 ml of Scintisol (Isolab).

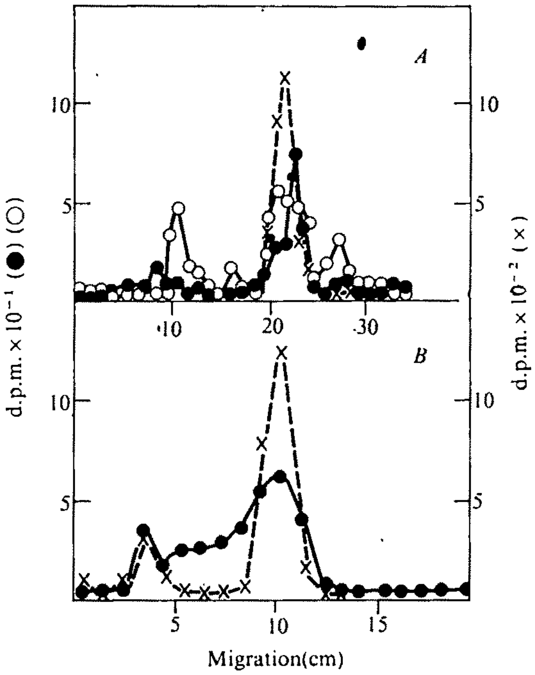

Fig. 3 Chromatographic and electrophoretic characterisation of acetyl peptides in the enzymatic digests. The water eluates from Dowex 50 H^+ columns (see Table 1) were flash evaporated and the residue resuspended in a small volume of water. Aliquots (10 µl) were analysed by (*A*) paper chromatography developed with *n*-butanol : acetic acid : water (12 : 3 : 5) and (*B*) paper electrophoresis[12]. Standard N-^3H-acetylserine was prepared[13] and included in each analysis. Paper strips (1 cm) were eluted with 1 ml of water for 1 h in counting vials, and the radioactivity was measured in a liquid scintillation counter with 10 ml of Scintisol. Solid circles and open circles represent values of nascent peptides labelled *in vitro* ^{14}C and *in vivo* ^3H, respectively. X represents N-^3H-acetylserine standard.

The experiments described here demonstrate that histone-like nascent peptides can be acetylated *in vitro* on polyribosomes from regenerating rat liver. They also show that most of the acetyl groups are attached to N-terminal amino acids, in particular to the amino group of serine. These observations can account for the *in vivo* acetylation of nascent peptides in polyribosomes from regenerating rat liver and provide direct evidence for a mechanism that can explain the presence of N-terminal acetyl groups in liver proteins, without the necessary involvement of N-acetylation during the initiation of protein synthesis[5]. The acetyltransferase activity found in rat liver ribosomes[6,9] may be the enzyme involved in the N-terminal acetylation of nascent peptides observed in this study. Since relatively few proteins are known to have N-acetyl termini, this enzyme must have a relatively high specificity. Further studies will be required to define this specificity in terms of the terminal sequence and the minimal size of the peptide substrate. Enzyme activities able to acetylate haemoglobin[14] or histone[15] have been found in ribosome-free reticulocyte lysates and rat liver postmicrosomal supernatants respectively. To what extent these activities are functionally involved in the N-terminal acetylation of proteins deserves further study.

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Spontaneous Re-formation of a Broken Peptide Chain

BASIC pancreatic trypsin inhibitor (BPTI) is a protein of fifty-eight amino acid residues, which inhibits the serine proteinases trypsin, chymotrypsin, plasmin and kallikrein. The sequence has been fully elucidated[1] (Fig. 1) and the three-dimensional structure has been determined to high resolution by X-ray crystallography[2,3]. In connection with studies on the partial synthesis of analogues of BPTI for the investigation of protein folding, we have examined in detail cleavage of the protein by cyanogen bromide[4]. We have found that the chain-cleaved product is unstable, and that on standing it is spontaneously transformed into an analogue of BPTI containing an intact chain of fifty-eight amino acid residues.

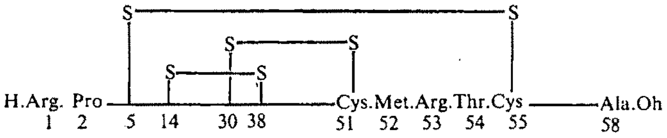

Fig. 1 BPTI part structure.

The single methionine residue in BPTI occurs at position 52 of the peptide chain. The crystal structure indicates that it is approximately in the middle of the only extended region of α-helix, and lies near the surface of the tightly folded molecule. Chemical evidence[5] indicates that the methionyl side chain is accessible to some, but not all, modifying reagents when the molecule is in its native conformation. There are also six cysteinyl residues in BPTI, which form three disulphide bridges. Two of these residues also occur in the α-helical region, at positions 51 and 55, straddling the position of the methionine residue. Thus, when the peptide chain of the native molecule has been broken with conversion of the methionine to homoserine lactone by cyanogen bromide, the resulting two backbone fragments are still held together by a disulphide linkage (between residues 5 and 55). As we shall demonstrate, this covalent bond between the fragments promotes the resynthesis of a peptide bond between residues 52 and 53 by attack of the newly generated amino terminus on the lactone carbonyl.

Treatment of BPTI with a hundred-fold excess of cyanogen bromide caused conversion of methionine to homoserine in 80% of the molecules within 20 h. Only one of the various expected products of cyanogen bromide treatment, the species in which chain cleavage between residues 52 and 53 has occurred and in which the homoserine residue is in the lactone form, should differ in net charge at neutral *p*H from BPTI. Accordingly ion-exchange chromatography of the cyanogen bromide-treated protein resolved two major peaks (Fig. 2). The first peak emerged at the same position as native BPTI; amino acid analysis of this material indicated that it contained all of the molecules with residual methionine. The second peak contained protein (II) in which the peptide chain has been broken. No methionine could be detected in this product, but acidic hydrolysates which had been heated in pyridine acetate just before analysis[8] contained 1.0 residue of homoserine per protein molecule. Furthermore, the new N-terminus, arginine 53, could be released in high yield by digestion of II with leucine aminopeptidase[9]. Although the normal N terminus of native BPTI is also arginine, this residue is not released by leucine aminopeptidase, presumably because residue 2 of the protein is proline.

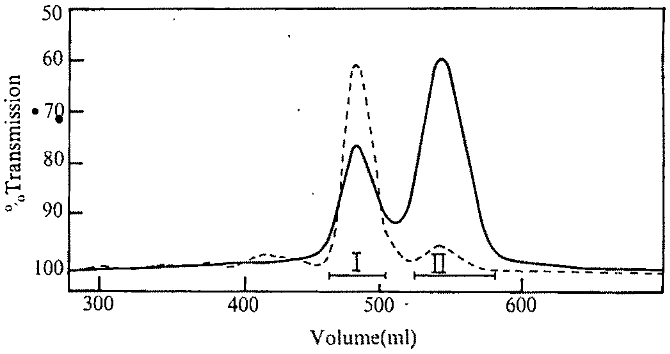

Fig. 2 (——), Ion-exchange chromatogram on a 1.3 × 30 cm column of carboxymethylcellulose (CM-52) of the desalted product from treatment of native BPTI with a hundred-fold excess of CNBr in 70% formic acid. The protein was chromatographed at *p*H 6.2 in 0.01 M phosphate buffer using a linear gradient of NaCl[6,7] (0–0.5 M, 400 ml in each chamber.) The position of peak I is identical to that obtained for native BPTI. Peak II corresponds to a more positive species. (- - -), Rechromatography of a sample of II after 6 d at *p*H 6.5.

Tryptic maps of II also definitely established backbone cleavage. When BPTI was reduced and carboxymethylated with ^{14}C-labelled iodoacetic acid, the resulting product was readily digested by trypsin[10,11], and electrophoresis at *p*H 6.5 resolved the four radiolabelled peptides (Fig. 3*a*). Two of these contained two carboxymethylcysteine residues each: peptide 1–15 (including cysteine residues 5 and 14) and peptide 27–39 (including cysteine residues 30 and 38). The other two peptides, 47–53 and 54–58, contained only one radiolabelled residue each, numbers 51 and 55, respectively. When II was reduced and carboxymethylated in the same manner, the hexapeptide 53–58 was removed upon desalting through Sephadex G25. Tryptic digestion of the large protein product and subsequent electrophoresis showed that the normal peptides 47–53 and 54–58 were virtually absent, while a new, more acidic peptide was present (Fig. 3*b*). This new peptide had an amino acid composition consistent with sequence 47–52 (52 = homoserine).

The properties of II have been observed to change with time in a manner which indicates resynthesis of the peptide backbone. This process has been primarily studied through the tryptic peptides of ^{14}C-carboxymethylated material. These studies were made quantitative by adding uniformly ^{3}H-carboxymethylated BPTI to samples before digestion, to act as an internal control. The ratio of ^{14}C to ^{3}H in the peptides 47–53 and 54–58 relative to that in the peptides 1–15 and 27–39 indicates the fraction of material in which the 52–53 peptide bond has been re-formed. For preparations of II in which the ion-exchange chromatography was performed at room temperature and the products were left at *p*H ~6.2 for a few hours, the amount of resynthesised protein varied from 15 to 35%. When chromatography was performed at 4° C and the products were trapped at approximately *p*H 3 by precharging each fraction with a small amount of acetic acid, the concentrations of peptides 47–53 and 54–58 detected could be kept to 5%.

The observations above are fully consistent with spontaneous resynthesis of the peptide chain in neutral solutions, and we have been able to follow the course of the resynthesis reaction with this radiolabelling procedure. A solution of II kept at *p*H 6.3 was sampled over the course of 2 d, and the described mapping procedure was employed at each stage. There was a progressive increase in the relative proportion of the peptides 47–53 and 54–58 and a corresponding attenuation in the amount of peptide 47–52 detected in successive samples, thereby indicating clearly the reconstitution of the protein backbone with time. Quantitative analysis of these successive samples by the method of double-counting outlined previously indicated an initial half-life for the resynthesis reaction of about 30 h. Similar analysis of a sample of II which had been reduced and carboxymethylated after standing 4 d at *p*H 6.3 (Fig. 3*c*) indicated the presence of peptides 47–53 and 54–58 in at least 85% yield and only a small amount of peptide 47–52. When the product obtained from such a resynthesis reaction was rechromatographed on the ion-exchange column used to isolate II, the majority of the material emerged in the position corresponding to BPTI (Fig. 2).

Neutral solutions of II also gained in inhibitory potency as a function of time. Preparations of II were found to be only 50% as potent as native BPTI in inhibiting trypsin (as determined by the benzoylarginine *p*-nitroanilide assay)[12] and 30% to 50% as potent in inhibiting chymotrypsin (as determined by the acetyltryptophan ethyl ester assay)[13]. In both cases the rate of increase towards the level of activity of BPTI agreed well with the rate of resynthesis demonstrated by the radiolabelling experiments.

The resynthesised protein, [52-homoserine]-BPTI, has been found to possess between 85% and 95% of the inhibitory potency of BPTI towards both trypsin and chymotrypsin. Furthermore, in the acetyltryptophan ethyl ester assay, in which BPTI is slowly displaced from chymotrypsin by the substrate, solutions of [52-homoserine]-BPTI of equal initial inhibitory potency exhibited a rate of inhibitor displacement indistinguishable from that for BPTI. The binding constants to chymotrypsin for both molecules must therefore be virtually identical. This is not surprising, as residue 52 is far removed from the active site of the molecule[11], and oxidation of methionine-52 has previously been shown not to alter its inhibitory properties[5].

Experiments involving reduction of the disulphide bridges and their subsequent reoxidation also demonstrated the resynthesis of the protein backbone in II. Native BPTI, which had been fully reduced in strong denaturing agents, could be reoxidised in neutral *p*H buffers by air or disulphide reagents with full regain of inhibitory activity. This was not true of II, which after reduction would regain activity only to the extent to which there was resynthesised material present. Nor would such samples regain activity beyond the level indicated when a ten-fold excess of the cleaved hexapeptide was added to the oxidising solution. Purified samples of [52-homoserine]-BPTI, however, were readily reoxidised to a fully active state after reduction. The rate of reoxidation of reduced [52-homoserine]-BPTI by disulphide reagents, measured as its rate in regaining potency as a trypsin inhibitor, was comparable to that measured simultaneously for reduced BPTI.

Our discovery of an example of facile resynthesis of a peptide backbone in a cyanogen bromide-cleaved protein indicates that this may be a practical route to the partial synthesis of some protein analogues. The direct coupling of lactone and amino components under favourable circumstances, as in this case where the fragments are held close together by a disulphide bridge, may provide routes for partial synthesis complementary to those already suggested employing such lactone functions[15], but which require more lengthy synthetic routes. We are at present exploring methods of utilising this more direct resynthesis reaction *en route* to new analogues of BPTI.

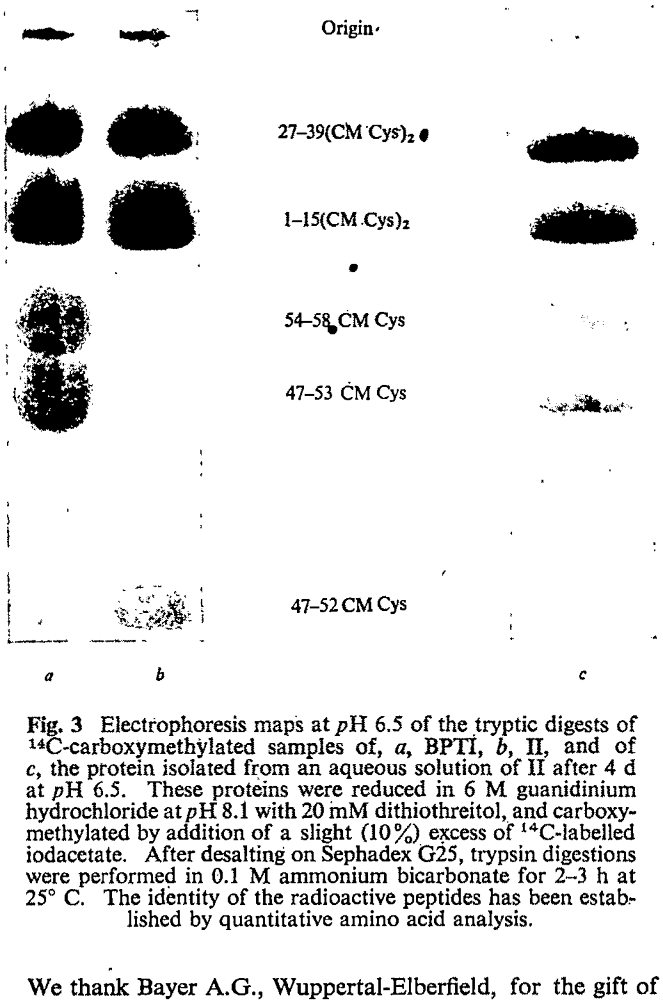

Fig. 3 Electrophoresis maps at *p*H 6.5 of the tryptic digests of ^{14}C-carboxymethylated samples of, *a*, BPTI, *b*, II, and of *c*, the protein isolated from an aqueous solution of II after 4 d at *p*H 6.5. These proteins were reduced in 6 M guanidinium hydrochloride at *p*H 8.1 with 20 mM dithiothreitol, and carboxymethylated by addition of a slight (10%) excess of ^{14}C-labelled iodacetate. After desalting on Sephadex G25, trypsin digestions were performed in 0.1 M ammonium bicarbonate for 2–3 h at 25° C. The identity of the radioactive peptides has been established by quantitative amino acid analysis.

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Structure and Conformation of a Cyclic Tripeptide

MANY cyclic peptides have interesting biological functions and the details of their molecular structure and conformation have been the subject of extensive investigations. Cyclic dipeptides such as diketopiperazine have been synthesised and shown to occur with the peptide units in the *cis* configuration[1,2]. In the case of a tripeptide, cyclisation can take place only if all three units are in the *cis* configuration[3]. In cyclic peptides with four units also, *cis* peptides are found[4,5]. As the number of the peptide units increases, the more stable *trans* configuration is generally more common[6,7]. We report here the main results of our X-ray crystallographic investigations of the cyclic tripeptides L-Pro-L-Pro-L-Pro and L-Pro-L-Pro-L-Hyp (hereafter called CTP 1 and CTP 2, respectively). CTP 1 was synthesised by Rothe *et al.*[8] and its derivatives have been prepared by Blout and his collaborators[9].

Venkatachalam[3] predicted that in the cyclic tripeptide CTP 1 all three peptides should be in the *cis* configuration for ring closure to be achieved. In his study, however, the conformational parameters were worked out on the assumption that the proline rings and the nitrogen environment are essentially planar. Nuclear magnetic resonance (NMR) studies on cyclic peptide derivatives have shown that the peptide units are indeed *cis* planar, but the proline rings are non-planar with the nitrogen atom out of the mean plane through the remaining four atoms of the proline ring[9]. Our studies show that the peptide units are *cis* planar in general, but the conformation of the proline rings is somewhat different from that reported in the NMR studies.

Crystals of CTP 1 and CTP 2 were recrystallised from material given to us by Professor E. R. Blout of Harvard Medical School. The crystal data are shown in Table 1. The diffraction data were collected manually on a GE XRD-3 diffractometer to a limit of $2\theta = 150°$. These two acentric structures, with 42 and 22 non-hydrogen atoms respectively in the asymmetric units, were solved by the direct methods using the MULTAN program[10], and refined by the block diagonal least squares method. During the refinement of CTP 2, it became apparent that the hydroxyl group was disordered. The electron density difference map showed two positions OH (1) and OH (2) for the hydroxyl group, the relative occupancy of OH (2) being twice the other. It is interesting that, for both these sites, the OH group is linked by a strong hydrogen bond (2.7 Å) to the carbonyl oxygen of a neighbouring molecule. All the hydrogen positions in the molecule of CTP 2 were established from the electron density difference map. The diffraction data from CTP 1 were of poor quality, and consequently unambiguous location of the hydrogen atoms was not possible in this case. A view of the molecule of the tripeptide as it occurs in the crystal structure of CTP 2 is shown in Fig. 1.

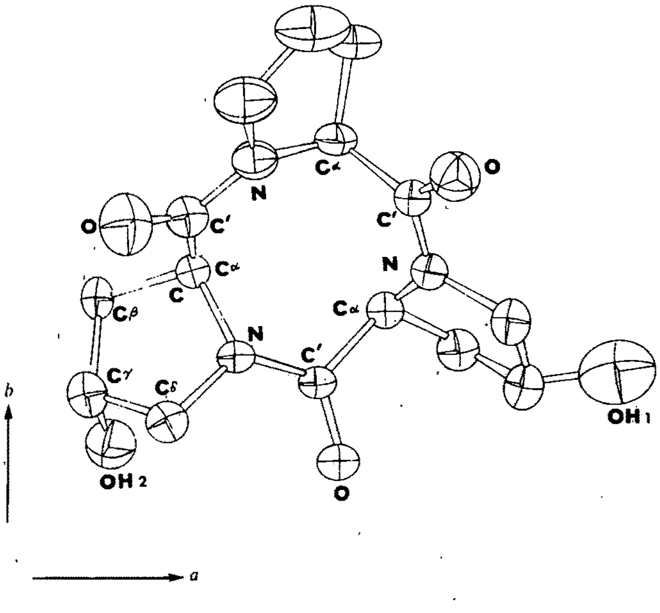

Fig. 1 View of the tripeptide molecule in CTP 2 looking down the *c* axis[13].

Table 1 Crystal Data of CTP 1 and CTP 2

	CTP 1	CTP 2
a	15.95 Å	16.28 Å
b	19.09 Å	9.45 Å
c	9.23 Å	9.23 Å
z	8	4
Space group	$P\,2_12_12_1$	$P\,2_12_12_1$
λ	1.5418 Å	1.5418 Å
Number of measured reflections	3,299	1,762
Percentage used for electron density calculation	79	97
Reliability index	0.13	0.06

The orientation of the tripeptide molecules in the structures of CTP 1 and CTP 2 is very similar when viewed down the *c* axis. The two molecules (*A* and *B*) in the asymmetric unit of CTP 1 are inclined to each other by about 55°. The average torsion angle $\chi_1(\text{N-C}_\alpha\text{-C}_\beta\text{-C}_\gamma)$ for the eight residues was 31°, in good agreement with the value obtained from NMR data[9]. The puckering of the different proline residues in the three tripeptide molecules is given in Table 2. The average value of the torsion angle $\omega(\text{C}_2\text{-C}'\text{-N-C}_2)$ is 1.8° for eight of the nine residues, while in the hydroxyproline residue it is as high as 19°. These torsion angles showed a fairly wide spread of about 5°. Therefore it seems that the cyclisation of three peptides could be achieved without invoking any excessive non-planarity of the peptide group. The backbone dihedral angles ϕ and ψ range from $-94°$ to $-110°$ and 83° to 98° respectively. These are appreciably different from the values $-78°$ and 109° predicted earlier[3]. Because of these variations in the torsion angles, from peptide to peptide in the tripeptide molecule, the backbone conformation in the crystal does not possess a true three-fold symmetry, assumed in interpreting the results of the solution studies[9]. Thus, we find many new types of conformation for the proline rings here different from any of those reported before in X-ray and NMR studies[11].

We have averaged the latest data from eight crystallographically independent *cis* proline residues in the crystals of CTP 1 and CTP 2 and obtained the dimensions of the standard *cis* proline residue (Fig. 2). The average ring torsion angles $\chi_1(\text{N-C}_\alpha\text{-C}_\beta\text{-C}_\gamma)$, $\chi_2(\text{C}_\alpha\text{-C}_\beta\text{-C}_\gamma\text{-C}_\delta)$, $\chi_3(\text{C}_\alpha\text{-C}_\beta\text{-C}_\delta\text{-N})$, $\chi_4(\text{C}_\gamma\text{-C}_\delta\text{-}$

Table 2 Puckering of Proline Residues in the Three Tripeptide Molecules

Atom out of plane	No. of residues CTP 1(*A*)	CTP 1(*B*)	CTP 2
C_α	1	—	1
C_β	2	3	1
N	—	—	1

N-C_α) are also shown in Fig. 2. It is worth noting that in CTP 1 and CTP 2 the C′-N bond distance (1.362±0.015 Å) is significantly larger than the value (1.32 Å) found in the *trans* peptide unit postulated by Corey and Pauling[12]. The average parameters we have obtained agree with the values reported by Karle[2].

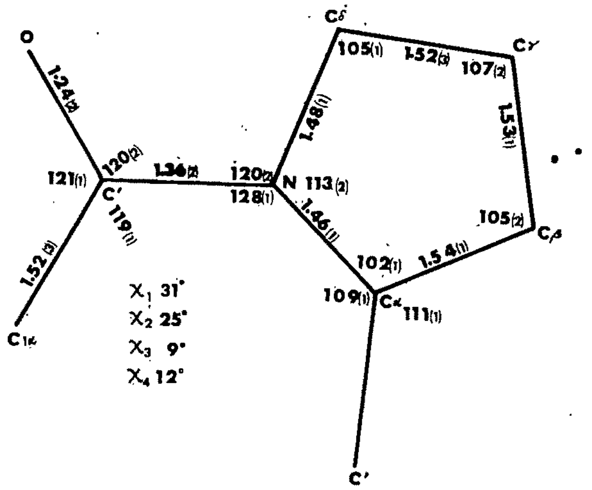

Fig. 2 Dimensions of the standard *cis* proline residue averaged from eight independent residues in CTP 1 and CTP 2. The RMS deviation in the dimensions in the eight residues is also indicated.

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Interferonogenicity and Antigen Recognition

NOT only viral and syngeneic polynucleotides but also bacterial endotoxins, PHA (refs 1 and 2), heterologous and, in certain cases, homologous gammaglobulins can induce interferon[3-5]. The question arises whether or not antigenicity correlates with interferonogenic properties. We therefore studied the interferonogenic properties of syngeneic and allogeneic living or irradiated mouse spleen cells (SC), xenogeneic and allogeneic erythrocytes and syngeneic materials (erythrocytes and sera) that had become autoantigenic after treatment with trypsin, iodination or heating.

SC were obtained from aseptically removed mouse spleens by soft compression in the glass Potter homogeniser. The cells were twice washed with medium 199 by centrifuging at 2,000 r.p.m. for 15 min, and the number of living nucleated cells was counted. Iodinated serum proteins were prepared according to Wormall[6]. The activity of serum interferon

Table 1 Interferonogenicity of Normal SC and Irradiated SC (ISC) for Syngeneic and Allogeneic Recipients*

Experiment No.	Recipient mice	Material injected	Time after injection (h)	Interferon activity† (units ml^{-1})
1	DBA/2	SC–Balb/c	6	0
	DBA/2	SC–Balb/c	12	60
	DBA/2	SC-Balb/c	24	50
	DBA/2	SC–Balb/c	48	20
	DBA/2	SC-DBA/2	6	0
	DBA/2	SC–DBA/2	12	0
	DBA/2	SC–DBA/2	24	0
	Balb/c	SC–Balb/c	12	0
	Balb/c	Without injection	12	0
	DBA/2	Without injection	12	0
2	F_1(CBA × C57Bl/6j)	SC–F_1(CBA × C57Bl/6j)	12	0
	F_1(CBA × C57Bl/6j)	SC–C57Bl/6j	12	5
	F_1(CBA × C57Bl/6j)	ISC–C57Bl/6j	12	0
	F_1(CBA × C57Bl/6j)	Without injection	12	0
3	C57Bl/6j	SC–Balb/c	16	70
	C57Bl/6j	SC–Balb/c	43	40
	C57Bl/6j	ISC–Balb/c	16	30
	C57Bl/6j	ISC–Balb/c	43	10
	C57Bl/6j irradiated*	SC–Balb/c	16	20
	C57Bl/6j irradiated	SC–Balb/c	43	0
	C57Bl/6j irradiated	ISC–Balb/c	16	5
	C57Bl/6j irradiated	ISC–Balb/c	43	0
	C57Bl/6j irradiated	Without injection	16	0
	C57Bl/6j irradiated	Without injection	43	0
4	C57Bl/6j	SC-DBA/2	4	10
	C57Bl/6j	SC–DBA/2	17	40
	C57Bl/6j	ISC–DBA/2	4	5
	C57Bl/6j	ISC–DBA/2	17	30

* SC exposed to gamma-irradiation, total dose 16,000 r, and mice recipients C57Bl/6j (in experiment 3), in a dose (total) of 850 r.

† Unit of interferon activity is a reciprocal of the endpoint serum interferon dilution inhibiting cytopathic effect.

was evaluated by a standard method[7]: 1 ml of the corresponding dilution of the serum to be studied (interferon) was placed on 48 h cultures of mouse L cells in each tube. The serum was removed 18–24 h later and 100 tissue culture pathogenic dose 50% ($TCPD_{50}$) of vesicular stomatitis virus were added and 24 h later the highest dilution of interferon that inhibits the cytopathic effect by 50% was determined. The tested dilutions of mouse sera higher than 1 : 20 were not toxic to L cells. In each experiment the interferon was titrated repeatedly two or three times. Mice of the strains Balb/c, DBA/2, C57Bl/6j and hybrids F_1 (CBA×C57Bl/6j) were used. Groups of fifteen to twenty-five mice recipients were inoculated with the following antigens: 10^8 of SC in 0.8 ml of medium 199, 6×10^9 erythrocytes in 0.5 ml of medium 199 and sera treated in different ways, in a volume of 0.5 ml. At different times (from 3 to 48 h) after antigen injection blood was taken from the mouse retro-orbital sinus and interferon was determined in the serum.

Intravenous injection of allogeneic SC leads to a marked interferon production in mice (Table 1). The maximum interferon concentration in the serum of mice recipients was observed 12–24 h after SC injection. Such interferon production takes place even in cases in which a donor and a recipient differ from each other in weak histocompatibility loci: mouse strains DBA/2 and Balb/c (experiment 1, Table 1). An intravenous injection of syngeneic SC does not lead to interferonogenesis. From experiments 2 and 3 with F_1 hybrids and with irradiated SC and/or irradiated recipients (see Table 1), it is evident that both donor and recipient lymphoid cells produce interferon.

Table 2 Interferon Activity after Injection into Mice of Normal and Trypsin-treated Red Blood Cells (RBC) and Iodinated and Heated Sera

Experiment No.	Recipient mice	Material injected	Time after injection (h) 6	12	24
5	C57Bl/6j	RBC–C57Bl/6j	0	0	0
	C57Bl/6j	RBC–Balb/c	5	5	5
6	DBA/2	RBC–Balb/c	—	0	—
	DBA/2	RBC–DBA/2	—	0	—
7	C57Bl/6j	RBC–sheep	5	5	5
	C57Bl/6j	Trypsin-treated RBC–C57Bl/6j	5	10	20
	C57Bl/6j	C57Bl/6j serum	—	—	0
	F_1(CBA × C57Bl/6j)	Syngeneic serum	—	0	—
	F_1(CBA × C57Bl/6j)	Iodinated syngeneic serum	—	20	—
	F_1(CBA × C57Bl/6j)	Syngeneic serum heated at 56°	—	5	—

This serum interferon was stable at 24 h at *p*H 2, lost antiviral activity in the presence of 0.1% of trypsin at 37° C for 1 h, failed to sediment at 100,000*g* for 1 h, did not lose activity when cultures treated with the serum interferon were washed before virus challenge, was active in mouse L cells against Venezuelan equine encephalitis virus and Sindbis virus, and did not possess any antiviral properties in the continuous cell culture of human amniotic F1 cells.

Allogeneic and xenogeneic erythrocytes induce only slight interferon production. Non-interferonogenic syngeneic erythrocytes acquire interferonogenic activity when treated with trypsin (Table 2). Syngeneic mouse serum is non-interferonogenic. The same serum becomes interferonogenic after heating or iodination.

In this laboratory the role of interferon in lymphocyte-target cell interaction has been established[8–11]. Interferon induces the sensitivity of target cells to the cytotoxic effects of normal allogeneic lymphocytes[12]. Other data on the connection of immune response and interferonogenesis have accumulated. Lymphoid cells were established as the main interferon producers *in vivo*[1,2]. A variety of agents suppressed antibody formation and interferon production in parallel[1]. Green *et al.*[13] suggested that immune recognition mechanisms are involved in enhanced interferon production in secondary immune response to non-viral antigens.

The experimental evidence presented above shows that cells and substances immunologically recognisable as 'self' do not induce interferon production and conversely cells and substances recognisable as 'non-self' do induce interferonogenesis after primary administration. The quantities of interferon produced after injection of various antigens were different. We suggest that antigen induces interferon production in cells involved in the first stage of the immune response.

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Spontaneous Fusion of Rat Liver Lysosomes *in vitro*

THE fusion of membranes is a key to the control of lysosomal function. Membrane fusion is involved in both the intra- and extracellular digestion of extracellular material (fusion between endocytotic vacuoles or between plasma membrane and enzyme-bearing vesicles) as well as in the formation of autophagic vacuoles. Primary lysosomes fuse readily with newly formed endocytotic vacuoles[1,2]; they seem to be able to fuse with heterolysosomes[3] but never directly with mitochondria or nuclei. Because of scanty information the apparent specificity in the fusion pattern of primary lysosomes cannot be explained in terms of relationships between membrane composition and function. Dingle[4] has proposed, in analogy to the coalescence of closed shells of fluid, or the 'breaking' of an emulsion by agglomeration of droplets to larger ones, that the fusion of cell membranes of high surface tension will be thermodynamically favoured. According to this suggestion lysosomes should be regarded as high surface tension vesicles.

The above considerations prompted us to investigate the possibility of spontaneous fusion of a lysosomal population *in vitro*. Lysosome-rich suspensions were incubated at 0° C and at 37° C for time intervals extending up to 3.5 h. At zero time, 0.5, 1 and 3.5 h the suspensions were analysed for free and 'available' acid phosphatase activity (Fig. 1), and

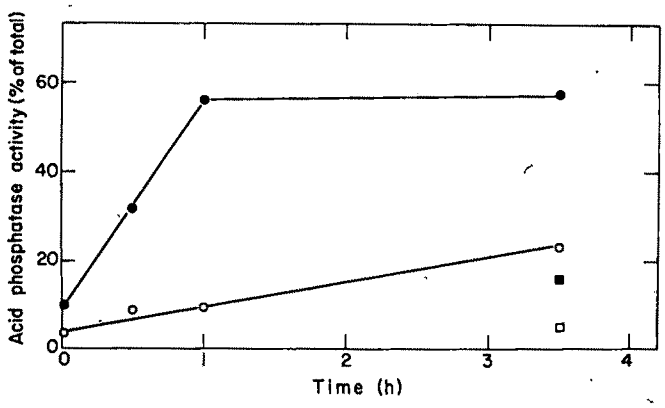

Fig. 1 Time dependence of free (○—○) and available (●—●) acid phosphatase activity in lysosomal suspensions incubated at 37° C. Corresponding values obtained in lysosomal suspensions incubated for 3.5 h at 0° C are also included (□, ■). Incubation mixture consisted of 23.5 mg of lysosomal protein ml^{-1} isolated as described[13], 0.25 M sucrose and 0.01 M Na-HEPES, *p*H 7. At the specified time intervals, 20 µl aliquots were removed from the suspensions and assayed[14] with *p*-nitrophenyl phosphate for free activity (detected in the medium after sedimentation of lysosomes), available activity (exhibited by the whole suspension), and total activity (reaction mixture made 0.1% in Triton X-100).

were fixed for electron microscopic examination. Continuous slow release of acid phosphatase to the medium was observed in lysosomal suspensions incubated at 37° C (20% in 3.5 h); no such release was observed in suspensions incubated at zero degrees. The latency of acid phosphatase activity was, however, markedly affected during incubation at 37° C. After about 1 h of incubation the available acid phosphatase activity of lysosomal suspensions increased from a value of 10% to about 60% of the total activity. The lysosomal membranes thus seem to have undergone a change in their barrier properties towards *p*-nitrophenyl phosphate, a substrate for acid phosphatase. No such change was observed in suspensions incubated at 0° C.

Electron micrographs of zero time suspensions showed populations of vesicles mostly regular in shape: spherical or ellipsoid. Some of the vesicles were more phosphotungstic acid positive, others were stained only in the limiting membrane and its close periphery (Fig. 2*a*). The same pattern was obtained in a histochemical assay of acid phosphatase (Fig. 2*b*). The dramatic change in both the dimensions and the shape of the suspended vesicles as a result of a 3.5 h incubation period at 37° C is shown in Fig. 2*c*. The average diameter of lysosomes incubated for 3.5 h at 37° C was twice that obtained for zero time control lysosomes (~0.5 µm). This indicates roughly at least a four-fold increase in vesicular surface area. Figure 2*d* shows lysosomes incubated for 3.5 h at zero degrees. It thus seems that the morphological changes are temperature dependent.

It is worth noting that lysosomal suspensions kept at 0° C (Fig. 2*a* and *d*) contain a significant percentage of mitochondria. No mitochondria could be detected in pellets of lysosomes incubated for 3.5 h at 37° C; suggesting mitochondrial breakdown, possibly due to the presence of free lysosomal enzymes in the medium.

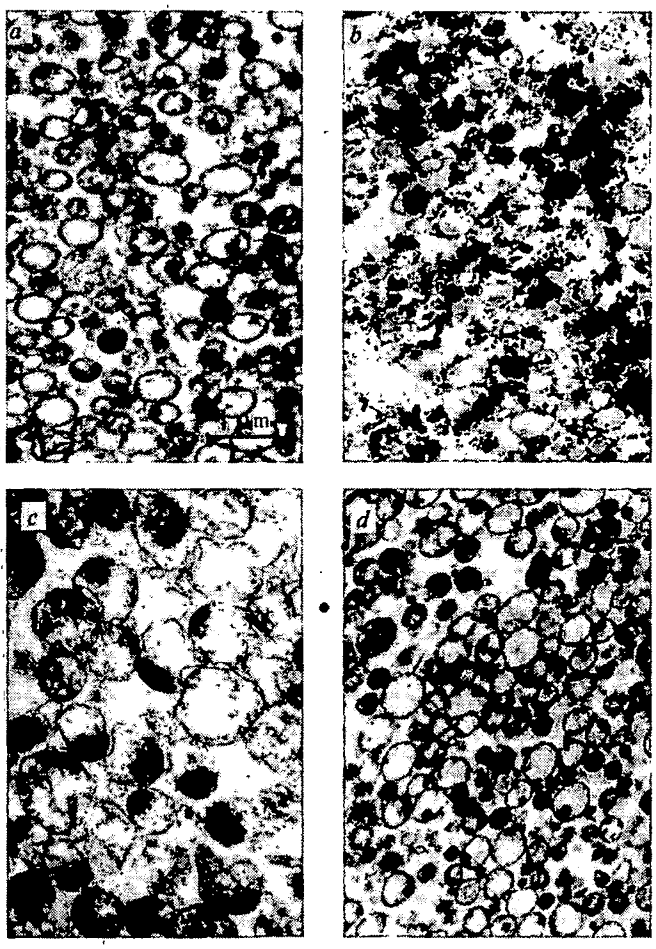

Fig. 2 Characteristic morphology of lysosome-rich suspensions, ×8,530. *a*, Zero time suspension; *b*, zero time suspension stained histochemically for acid phosphatase[15]; *c* and *d*, lysosomal suspensions incubated for 3.5 h at 37° C and at 0° C, respectively. Incubation of the lysosomal suspensions (described in Fig. 1) was terminated by centrifugation. The lysosomal pellets were resuspended in 0.1 M Na-cacodylate, *p*H 7.4, containing 2% of glutaraldehyde. After 1 h of fixation the pellets were washed, and post-fixed for 1 h with 1% osmium tetroxide. The resulting precipitate was dehydrated and embedded in epoxy resin according to Spurr[16]. Thin sections obtained with Sorvall, Porter-Blum MT 2-B ultra-microtome were stained for 30 s with 1% phosphotungstic acid in 70% ethyl alcohol, and were analysed in a Phillips 300 electron microscope.

The enlargement of vesicular contours could be brought about either by intralysosomal degradation of macromolecules, leading to an increase in osmotic pressure and lysosomal swelling or by fusion of vesicles. The first mechanism would imply that the lysosomal membrane has an unusual expanding capacity, since lysosomes are not endowed with biosynthetic capacity. Measurements of membrane thickness in both 0° C control and 37° C incubated lysosomes do not support such a mechanism. Fusion of vesicles during the incubation period at 37° C is the most plausible explanation for the morphological transformation of the lysosomal suspension. Since 3.5 h incubation yielded a rather homogeneous population of enlarged vesicles, it could be reasoned that on shorter incubation intermediate steps in the fusion process could be arrested. Figure 3 shows such intermediate steps. Note the small protrusions which develop at areas of close contact between lysosomes (Fig. 3*a* and *b*). It would seem reasonable that these protrusions facilitate the interdigitation of membrane elements of two vesicles. A latter step involving membrane fusion would establish permanent connections between vesicles. The resulting structures seem to be closed vesicles encompassed by an intact membrane. Phosphotungstic acid positive material tends to aggregate at one pole of the vesicles (Fig. 2*c*). As previously pointed out the observed two-fold increase in particle diameter indicates for a sphere a four-fold increase in surface area and an eight-fold increase in volume. If it is assumed that the total membrane area is constant and that intralysosomal volume is preserved (no swelling following fusion), then it is to be expected that the vesicles resulting from lysosomal fusion would not be spheres. This indeed seems to be the case (see Fig. 2*c*). The continuous release of acid phosphatase to the suspending medium (Fig. 1) suggests that some lysis accompanies lysosomal fusion. Such a process has been proposed as a prerequisite to chemical[5,6] and viral[7] induced fusion of cells.

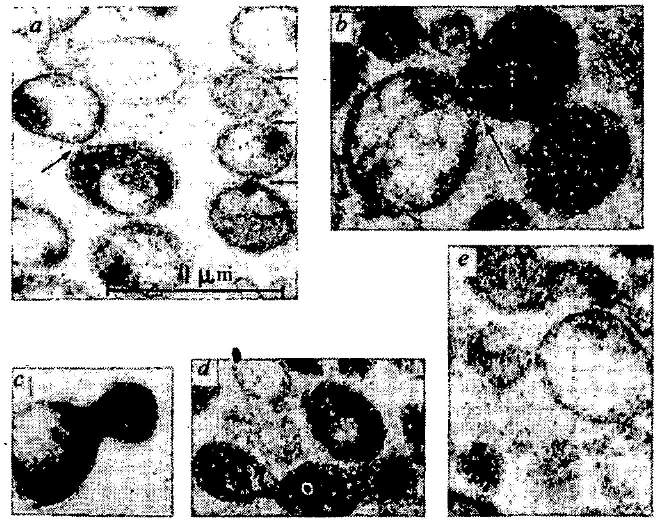

Fig. 3 Intermediate steps in lysosome fusion. Lysosomal suspensions (details in legend to Fig. 1) were incubated for 0.5 h (*a* and *c*) and for 1 h (*b*, *d*, *e*) at 37° C. Incubation was terminated by centrifugation and the pellets processed as described for Fig. 2. *a*, ×22,500; *b*, *c*, ×67,500; *d*, *e*, ×29,160.

The observed temperature dependence of fusion could be attributed to the involvement of enzyme action, though chemically induced cell fusion[8] has also been shown to be markedly temperature dependent.

The molecular events preceding fusion are not yet understood. It seems, however, that extraorganelle energy sources or bivalent cations are not essential elements in the process. It has been suggested[9] that a primary requirement for the fusion of two biological membranes is that both membranes involved have a relatively high proportion of their phospholipid molecules in the micellar configuration. It was also argued that molecules like lysolecithin, found in lysosomal membrane phospholipids[4], can induce micellar configuration[9]. Moreover, the presence of phospholipases in lysosomes which could degrade lecithin to lysolecithin has been reported[10,11].

Similar morphological changes, that is, the appearance of protrusions leading to bridge formation, have been reported in the case of fusion of rat parotid secretory granules with the cell membrane at the lumen[12] and in chemically induced fusion of avian erythrocytes[5].

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Selective Binding Properties of Vitamin D Transport Protein in Chick Plasma *in vitro*

WHILE vitamin D$_2$ and vitamin D$_3$ are equipotent antirachitic agents in most mammals[1] ergocalciferol (D$_2$) is approximately 1/100 as potent as cholecalciferol (D$_3$) in preventing or curing rickets in young chicks[2]. Although the basis of the insensitivity in chicks to D$_2$ has not yet been elucidated, it had been noted that radioactive vitamin D$_2$ is cleared from blood more rapidly than radioactive D$_3$ while inactive metabolites of D$_2$ are excreted more rapidly in the bile and faeces[3]. We have shown that a specific transport protein for vitamin D and D metabolites exists in the plasma of D-deficient chicks as well as in the plasma of several mammalian species[4]. These specific transport proteins serve to solubilise vitamin D compounds in the aqueous environment of plasma and to protect the steroids from oxidative inactivation[5]. Since vitamin D requires efficient plasma transport to the liver and the kidney for successive enzymatic hydroxylations[6–8] and the 'activated' metabolites must, in turn, be transported efficiently to the target tissues, we undertook studies to test the hypothesis that the lower potency of D$_2$ might, in part, reflect weaker binding of vitamin D$_2$ and its metabolites to the chick plasma transport protein.

Our approach to this problem involved the use of D$_2$ and D$_3$ analogues to competitively displace ^{3}H-25-OH-D$_3$ from the specific binding protein in plasma from various species[9]. It was necessary to first remove all non-specific binding proteins such as chylomicra, low density and very low density lipoproteins from the plasma through selective precipitation with heparin and manganese chloride[10]. The plasma was then diluted with barbital acetate buffer, *p*H 8.6 (ref. 9), and used in an assay system which included ^{3}H-25-OH-D$_3$ and the vitamin D analogue to be tested. Following a 2 h incubation at 4° C, separation of the unbound ^{3}H-25-OH-D$_3$ from that associated with the specific binding protein was accomplished with dextran-coated charcoal and the ^{3}H in the supernatant was counted in Aquasol (New England Nuclear). Increasing amounts of each analogue tested produced progressive reduction of the ^{3}H-25-OH-D$_3$ in the 'bound' supernatant fraction allowing the development of displacement curves and comparison of the relative binding of the D$_2$ and D$_3$ analogues by the plasma from chick and other species.

In the assay system with rat plasma, increasing amounts of vitamin D$_2$ and D$_3$ produced similar reductions of the ^{3}H-25-OH-D$_3$ in the supernatant fraction (Fig. 1*a*) indicating equivalent binding of D$_2$ and D$_3$ by the rat transport protein. Increasing amounts of 25-OH-D$_2$ and 25-OH-D$_3$ also produced quantitatively equivalent reduction of ^{3}H-25-OH-D$_3$ confirming the equivalent binding of D$_2$ and D$_3$ by the rat binding protein for vitamin D (Fig. 1*b*). Equivalence of binding of D$_2$ and D$_3$ was also found with the transport proteins in human, rabbit, cow, and dog plasma, species in which D$_2$ and D$_3$ are biologically equivalent[1]. In contrast, using the assay

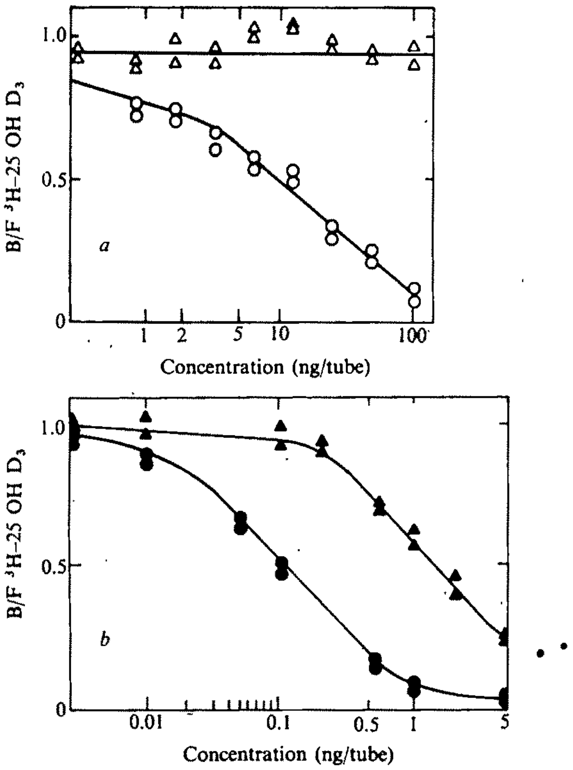

Fig. 1 Displacement of ^{3}H-25-OH-vitamin D_3 from D-deficient rat plasma with vitamin D analogues. Plasma obtained from D-deficient rats was cleared of non-specific binding proteins[10] and diluted with barbital acetate buffer, *p*H 8.6. Increasing amounts of the analogue to be tested with a fixed amount of ^{3}H-25-OH-D_3, all in 95% ethanol, were added to the diluted binding protein (B) or to buffer only (D). An additional aliquot of the analogue with the ^{3}H-25-OH-D_3 was added to a counting vial for determination of the c.p.m. added to each tube (T-total c.p.m.). Separation of unbound ^{3}H-25-OH-D_3 in both B and D tubes was accomplished with dextran-coated charcoal (0.25% Norit A Charcoal and 0.025% Dextran T80). Specific binding was determined by subtracting c.p.m. in the supernatant in the absence of binding protein (D) from the c.p.m. with the binding protein present (B) and the bound/free (B/F) ratio calculated: $B/F = B - D/T - (B-D)$. ○, D_3; △, D_2; ●, 25-OH-D_3; ▲, 25-OH-D_2.

system with chick plasma, increasing amounts of D_2 were ineffective in displacing the ^{3}H-25-OH-D_3 when compared with D_3 (Fig. 2*a*). Tests of displacement of the ^{3}H-25-OH-D_3 by concentrations of D_2 in excess of 100 ng per tube were not possible due to the limited solubility of the fat-soluble vitamin in the aqueous medium of the assay system. 25-OH-D_2 was less efficient than 25-OH-D_3 in displacing the ^{3}H-25-OH-D_3 from the chick transport protein (Fig. 2*b*); fifteen times more 25-OH-D_2 than 25-OH-D_3 (w/w) was required to produce 50% displacement. Similar results, that is, marked reduced binding of D_2 or 25-OH-D_2 by blood transport proteins, were found in the plasma of a number of chicks tested.

These findings suggest that resistance to the action of vitamin D_2 in the chick may be due, wholly or in part, to defective binding of D_2 and 25-OH-D_2 by the chick transport proteins resulting in lowered efficiency of the hepatic and/or renal hydroxylations of the vitamin and reduced delivery of the dihydroxy metabolites of vitamin D_2 to target tissues. Further investigation in other species known to be resistant to the action of vitamin D_2 will be important to establish whether similar correlations exist between biological activity and binding to the transport proteins. These results also suggest that selective measurement of vitamin D_3 and 25-OH-vitamin D_3 will be possible using the chick transport proteins, with little interference from D_2 or 25-OH-D_2. Simultaneous assays[9] using chick and rat plasma might then prove useful in distinguishing the relative concentrations of D_2 and 25-OH-D_2 (dietary sources) from D_3 and 25-OH-D_3 (sunlight exposure) in man and other species and, thereby, provide insight into the respective importance of environmental and dietary factors in supplying vitamin D requirements in man.

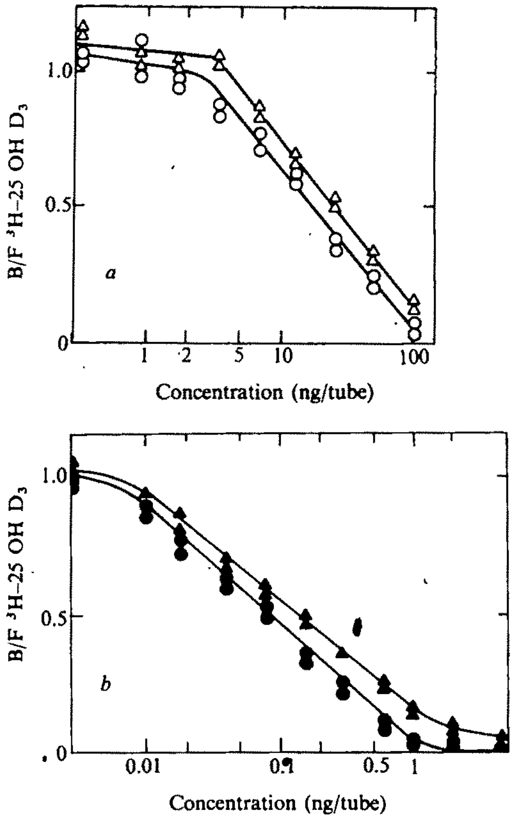

Fig. 2 Displacement of ^{3}H-25-OH-vitamin D_3 from D-deficient chick plasma with vitamin D analogues. The determination of B/F ratios for comparing binding of D analogues was accomplished with the procedure outlined in Fig. 1 except that chick plasma was used as a source of the specific D binding protein. Symbols as in Fig. 1.

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An Inhibitor of Sterol Biosynthesis present in Cow's Milk

RECENT evidence indicates that sterol biosynthesis from acetate, but not from mevalonate, increases several-fold in the developing rat liver after weaning[1]. This increase parallels the postnatal rise in activity of liver 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), an enzyme which seems to catalyse the rate-limiting key reaction of hepatic sterologenesis both in the adult and in the developing rat[2,3]. Delayed weaning blocks the normal increase of HMG-CoA reductase which indicates that dietary factors are responsible for the observed changes, and rat milk was shown to contain an inhibitor of HMG-CoA reductase activity[2].

Here we present evidence that cow's milk, an important component of human diet, exhibits an inhibitory effect on rat liver sterologenesis both *in vitro* and *in vivo*. All experiments were performed on 5 to 6-week-old male Wistar rats. The animals were killed at the same time of day, between 1100 and 1200 h. Defatted cow's milk (fat content 2%, protein content 3.5%) was obtained from state controlled commercial sources, human milk directly from the patients of the Obstetric Clinic. A standard diet for laboratory animals, 'Bacutil'-Poland, called in the table 'solid food', was used, except where stated otherwise. As shown in Table 1, 10 μl cow's milk or 25 μl of human milk added to 2.6 ml of incubation mixture inhibited completely the incorporation of ^{14}C-acetate and ^{3}H-mevalonate into digitonin-precipitable sterols by liver preparations. The inhibitory activity of cow's or human milk resisted 10 min boiling and was found in the supernatant after protein had been precipitated from cow's milk with 5% lactic acid or 5% trichloroacetic acid (TCA). Overnight dialysis against Bucher medium[4] completely abolished the inhibitory effect of cow's milk. These facts suggest a low molecular weight and probably non-protein nature for the sterol synthesis inhibitor. It should be noted that, in contrast to our findings, the inhibitory activity of rat milk on HMG-CoA reductase was found to be non-dialysable.

Table 1 Effect of Milk on the Incorporation of ^{14}C-Acetate and ^{3}H-Mevalonate into Digitonin-precipitable Sterols by 600*g* Supernatant of Rat Liver

Additions (μl)		Specific radioactivity of sterols			
		^{3}H d.p.m. mg^{-1}	% of control	^{14}C d.p.m. mg^{-1}	% of control
None (control)		39,400	100	10,000	100
Cow's milk	10	780	2	—	0
Cow's milk boiled	10	800	2	—	0
Human milk	25	1,900	5	—	0
Human milk boiled	25	2,000	5	—	0
Cow's milk dialysed	25	38,800	100	9,800	100
Supernatant of cow's milk deproteinised with lactic acid, final conc. 5%	25	750	2	—	0
Supernatant of cow's milk deproteinised with trichloroacetic acid, final conc. 5%	25	—	0	—	0
Neutralised lactic acid 5%	50	39,000	100	10,000	100
Neutralised trichloroacetic acid 5%	50	39,000	100	10,000	100

2.5 ml of 600*g* supernatant of 33% rat liver homogenate in 0.1 M potassium phosphate buffer, *p*H 7.4, 0.004 M $MgCl_2$, 0.001 M EDTA and 0.125 M sucrose 'Bucher medium'[4] was incubated at 37° C for 2 h with 1.0 μCi 1-^{14}C-acetate and 0.5 μCi 2-^{3}H-mevalonate in a total volume of 2.6 ml. Incubation was stopped by adding 15% KOH in methanol and the mixture was saponified at 65° C for 3 h with 4 mg of cholesterol added as carrier. The non-saponifiable lipids were extracted with hexane. The extracts were evaporated and digitonides were prepared according to Sperry and Webb[6]. The digitonides were dissolved in hot methanol and aliquots were taken for both radioactivity and sterol determinations. Sterols were assayed with the Liebermann-Burchardt colour reagent. The endogenous sterol content was 28 mg per 100 ml of supernatant fraction used. ^{3}H and ^{14}C radioactivity was analysed in a Nuclear Chicago Isocap 300 liquid scintillation counter using the scintillation fluid containing 100 mg POPOP and 4 g PPO per l toluene. The values represent the means of typical experiments run in triplicate.

Table 2. Effect of Dietary Cow's Milk on the Incorporation of ^{14}C-Acetate and ^{3}H-Mevalonate into Digitonin-precipitable Sterols by Rat Liver Slices

Group	Diet	Specific radioactivity of sterols	
		^{3}H d.p.m. mg^{-1}	^{14}C d.p.m. mg^{-1}
(1)	Solid food+drinking water (control)	4,470±580	15,400±1,270
(2)	Solid food+milk	4,600±420	7,300±1,290
(3)	Milk only	4,600±310	8,600±1,100

Each group consisted of three rats. The animals were kept for 3 d on the diet indicated. Daily intake of solid food in groups 1 and 2 was about 8.5 g per rat. Daily intake of milk was about 7 ml per rat in group 2 and 12 ml per rat in group 3. 0.5 g portions of liver slices obtained in duplicate from each animal were incubated at 37° for 2 h with 1.0 μCi 1-^{14}C-acetate and 0.3 μCi 2-^{3}H-mevalonate in 1.0 ml of Krebs-Ringer phosphate buffer, *p*H 7.4 (ref. 7). Incubation was carried out under continuous oxygen flow and was stopped by freezing. Tissue was separated from the medium and saponified at 65° C for 3 h in 5.0 ml of 15% KOH in methanol with 6.25 mg of cholesterol added as carrier. Further experimental details as in legend to Table 1. The endogenous sterol content was 2.2±0.2 mg per g of wet liver. The results of a typical experiment are presented. Values are the means from three animals±s.d.

To check whether cow's milk present in the diet could diminish the rate of hepatic sterologenesis, *in vivo* experiments were performed. As shown in Table 2, the incorporation of ^{14}C-acetate into sterols by liver slices obtained from rats fed milk diet for 3 d was about 50% lower than in the control. Sterol synthesis from ^{3}H-mevalonate, however, was not affected. It should be emphasised that cholesterol content in the standard diet was 0.36 mg g^{-1} and that defatted cow's milk used in our experiments contained about 8 mg of cholesterol per 100 ml. Since it is well established[5] that the feedback inhibition of hepatic cholesterogenesis by exogenous cholesterol requires at least 0.1% of cholesterol in the diet, it seems unlikely that the relatively small amount of cholesterol taken in with the milk would be the cause of the inhibitory effect observed in the rats of groups 2 and 3 (Table 2). Our conclusion is that a dialysable, TCA-soluble inhibitor of sterol synthesis exists in cow's milk. This inhibitor, although effective both *in vivo* and *in vitro*, affects sterol synthesis in a different way when added to the cell-free system than when fed to the intact animal. Whether the inhibitory effect on sterol biosynthesis from acetate and mevalonate is related to the same or different compounds present in milk remains to be solved. The properties and chemical nature of the milk inhibitor are under investigation.

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DNA Breaks in Rifampin-treated *Salmonella typhimurium* LT2 after Exposure to Nutritionally Complex Media

We have reported the phenomenon of "minimal medium recovery" of heated *Salmonella typhimurium* LT2 (ref. 1) and how this phenomenon could be explained in terms of DNA damage caused by sequential exposure to heat and nutritionally complex medium[2]. We now show that treatment of *S. typhimurium* with rifampin also results in decreased viability on rich relative to minimal medium and that the DNA from these rifampin-treated bacteria exhibits breaks after exposure to a rich or complex medium.

When exponentially growing cultures of *S. typhimurium* in a glucose-mineral salts medium (M-9)[1] were exposed to 20 μg of rifampin per ml of medium for 15 min, the viable counts on M-9 agar plates remained constant while viable counts on trypticase soy agar, enriched with 0.5% yeast extract (TSY), showed a 99.0–99.9% reduction (Fig. 1). When the rifampin was removed, the TSY plate counts increased to the same level as before rifampin treatment within 2–3 h. The increase in TSY plate counts indicates repair of the damage produced by rifampin treatment. This observation is similar to the behaviour of heated *S. typhimurium*, although the mechanisms leading to TSY sensitivity may be different for both stresses (heat and rifampin treatments).

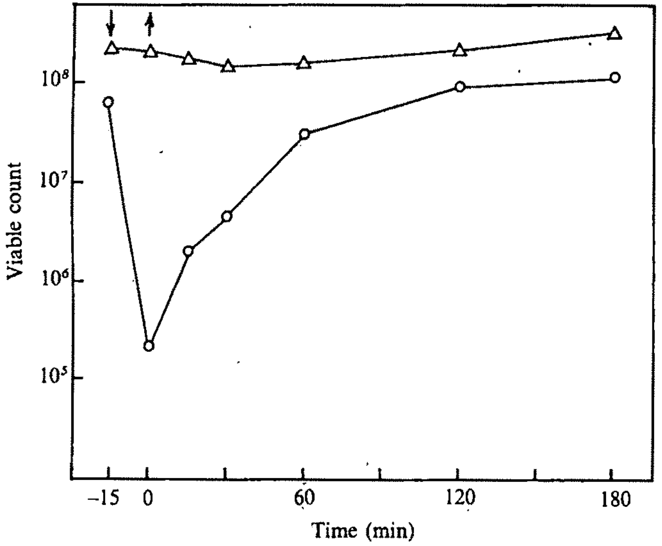

Fig. 1 Behaviour of *S. typhimurium* LT2 during and after incubation in M-9 with rifampin. An exponentially growing culture of *S. typhimurium* in M-9 broth was diluted (1 : 2) with M-9 broth containing 40 µg of rifampin per ml of broth. After 15 min of incubation at 37° C, 10 ml of broth was filtered on a Millipore membrane (HA 0.45 µm; 25 mm) and washed twice with 10 ml of M-9 medium prewarmed to 37° C. The filter was then resuspended in 10 ml of M-9 without rifampin and incubated at 37° C. Viability was assayed on M-9 (△) and TSY (○) plates. ↓, Addition of rifampin; ↑, removal of rifampin.

Since DNA from heated *S. typhimurium* exhibited breaks after exposure to TSY, we decided to repeat the same experiment with rifampin-treated bacteria. Radioactively labelled cells, grown in M-9 broth to the exponential phase, were treated with rifampin for 15 min and subsequently incubated in TSY for another 15 min. The cells were subjected to alkaline sedimentation analysis after rifampin and again after TSY incubation (Fig. 2*a*). The sedimentation patterns obtained show that rifampin treatment alone did not alter the weight-average molecular weight of DNA; but, when the rifampin-treated bacteria were exposed to TSY broth, the weight-average molecular weight was reduced ten-fold (Fig. 2*b* and Table 1). Control experiments demonstrated that exposure to TSY broth alone did not alter the sedimentation pattern of DNA. Likewise, as with heat, cells exposed to rifampin and then TSY lose viability when assayed on M-9 plates (Table 1) and also develop breaks in their DNA.

Table 1 Viability and DNA Weight-Average Molecular Weight of *S. typhimurium* LT2 after Incubation in M-9 with Rifampin followed by Incubation in TSY Broth

Treatment	Viable counts per ml M-9 plates	Viable counts per ml TSY plates	Weight-average molecular weight*
None	3.0×10^7	1.8×10^7	1.4×10^8
15 min in M-9+20 µg ml^{-1} RIF	4.0×10^7	4.8×10^5	1.4×10^8
15 min in M-9+20 µg ml^{-1} RIF followed by 15 min in TSY	2.0×10^6	2.0×10^5	1.2×10^7

RIF, rifampin.
* Computed from Fig. 2.

Figure 1 shows that rifampin-treated *S. typhimurium* recover their resistance to TSY if incubated in M-9 broth without rifampin. Table 2 shows that, in addition to the recovery of resistance to TSY broth, as assayed by viability on M-9 plates, DNA from rifampin-treated bacteria exhibited fewer breaks after incubation in TSY if they were previously incubated in M-9 broth. In other words, repair of rifampin-induced damage can be determined both by the ability of rifampin-treated bacteria to grow on TSY plates and by their resistance to TSY-induced DNA breakage.

Whether or not the reduced survival of rifampin-treated bacteria on TSY plates is a direct consequence of DNA breakage when these bacteria are exposed to TSY, it is interesting that rifampin-treated bacteria suffer DNA breakage when exposed to TSY. Bridges *et al.*[3,4], Woodcock and Grigg[5] and Sedgwick and Bridges[6] have proposed that DNA breaks after mild thermal treatments occur enzymatically; we concur. Along with Woodcock and Grigg[5], we propose that endonucleases recognise thermally denatured sections of the DNA molecule. Such enzymes have been generally reported to be present in *Escherichia coli* by Goldmark and Linn[7] and by Verly *et al.*[8]. In addition, we believe that these endonucleases are activated by TSY. It is then compelling to ask if and how rifampin renders the DNA molecule susceptible to nucleolytic activity.

We would like to advance the following model. It has been proposed[9] that transcription results in transient disruption of the DNA to DNA base pairing. Recently, it has also been shown[10] that *E. coli* RNA polymerase can bind to bacteriophage ϕX174 DNA replicative form and thus protect specific fragments from endonucleases. Since the addition of rifampin blocks initiation of transcription without affecting RNA chain elongation already in progress[11], it is possible that this situation could lead to transient regions of disrupted base pairing not protected by RNA polymerase. These regions would then be susceptible to endonucleases activated by TSY.

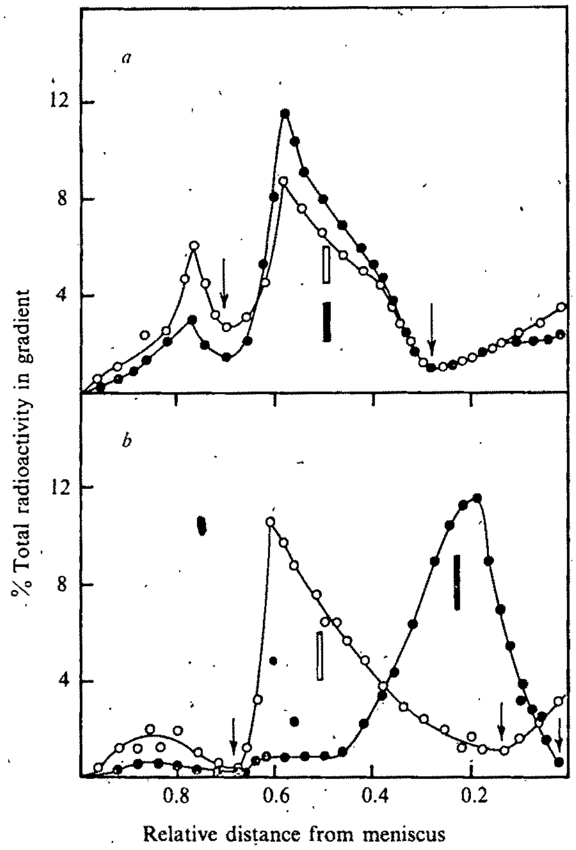

Fig. 2 Alkaline sedimentation of DNA from cells of *S. typhimurium* LT2 cultures incubated in M-9 with 20 µg rifampin per ml of broth at 37° C for 15 min followed by incubation in TSY for 15 min at 37° C. Cultures of *S. typhimurium* were grown to the exponential phase at 37° C in M-9 broth containing 250 µg of 2′-deoxyadenosine[13] and either 15 µCi of ^{3}H-thymidine-methyl (6.7 Ci mmol^{-1}, New England Nuclear Corp.) or 5 µCi ml^{-1} of ^{14}C-thymidine-methyl (51.5 mCi mmol^{-1}, New England Nuclear Corp.) of medium. ^{3}H-labelled cells were exposed to 20 µg of rifampin per ml of suspension for 15 min (*a*) and half of the cell suspension was then subjected to alkaline sedimentation analysis[2] along with half of the ^{14}C-labelled cells which were untreated and served as internal markers. The remaining half of the ^{3}H-labelled rifampin-treated cells were further incubated in TSY broth at 37° C for 15 min (*b*) and were then subjected to alkaline sedimentation analysis along with control ^{14}C-labelled cells. Centrifugation was done in a Spinco SW 50.1 rotor in a Beckman ultracentrifuge (L3–50) at 20° C for 40 min at 40,000 r.p.m. ○, ^{14}C-DNA; ●, ^{3}H-DNA. Arrows indicate limits within which DNA weight-average molecular weight was calculated[2]. Open and closed bars indicate position of DNA having the calculated weight-average molecular weight for ^{14}C and ^{3}H-labelled DNA respectively. Sedimentation is right to left. The fast sedimenting fractions in control and some of ^{3}H-labelled cultures are believed to be DNA-membrane complexes[14].

Rifampin inhibits RNA synthesis, not by blocking the initial binding of RNA polymerase to DNA[4,11], but more likely by inhibiting the transformation of the DNA•enzyme complex to an activated state, where enzyme and DNA change their conformation allowing the enzyme to bind to specific promoter sites on the DNA[12]. Then, if RNA polymerase protects DNA from TSY-activated nucleases, the protective form has to be the above mentioned activated RNA polymerase-DNA complex. Whether or not inhibition of RNA synthesis initiation with rifampin yields partially denatured DNA regions remains to be determined. It should also be mentioned that if rifampin intercalates between the bases of the DNA molecule, then this would also produce local denaturation. However, we do not know of any evidence that rifampin in fact intercalates between the DNA bases.

Table 2 Effect of Incubation of Rifampin-treated *S. typhimurium* LT2 in M-9 Broth Before Incubation in TSY Broth on Viability and DNA Weight-Average Molecular Weight*

Time in M-9 after rifampin treatment before TSY incubation (min)	Viability on M-9 after TSY incubation (cells ml^{-1})	Weight-average molecular weight after TSY incubation †
0	8.0×10^5	1.8×10^7
30	3.0×10^6	2.9×10^7
60	8.3×10^6	4.2×10^7
120	4.0×10^6	8.3×10^7
240	6.0×10^7	1.0×10^8
300	7.0×10^7	1.5×10^8

* Exponentially growing cells, as described in Fig. 2 legend, were exposed to 20 µg of rifampin per ml of M-9 broth for 15 min and were then washed free of rifampin and incubated in M-9 broth. At different times during M-9 incubation, portions of the cell suspension were exposed to TSY broth for 15 min at 37° C and were then subjected to alkaline sedimentation and viability analysis. Before rifampin treatment there were 6.8×10^7 viable cells per ml as assayed on M-9 plates.

† The DNA weight-average molecular weight before rifampin treatment was 1.5×10^8.

Indeed, although we have not shown that DNA breakage occurs through endonucleolytic attack, the alternative hypothesis of a physically-induced breakage is hard to visualise. It seems to us that both heat and rifampin cause a change in the cell such that, when exposed to nutritionally rich media, lethal DNA breaks ensue.

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Evidence for Individual Human Peripheral Blood Lymphocytes bearing both B and T Cell Markers

LYMPHOCYTES are composed of two main populations: thymus-derived (T) lymphocytes which are largely responsible for cell-mediated immunity, and bursal-equivalent or bone-marrow derived (B) lymphocytes which are primarily involved in humoral immunity. B cells can be identified by the presence of fluorescence-detectable surface immunoglobulin[1–3] and by their ability to bind antigen-antibody complexes or heat-aggregated immunoglobulin through the Fc portion of the immunoglobulin molecule[4–7]. Human T cells can be identified by their ability to form non-immune rosettes (E rosettes) with sheep red blood cells (SRBC)[8–12]. We report here that when these B and T cell markers were evaluated simultaneously on individual cells, a small subpopulation reproducibly bore both markers. Further experiments seemed to rule out artefact as an explanation.

Lymphocyte separation and fluorescent detection of surface Ig and aggregated immunoglobulin-binding were performed as before[5,6]. Briefly, peripheral blood lymphocytes (PBL) were isolated from venous blood by Ficoll-Hypaque density flotation followed by passage over nylon fibre columns. The purified PBL were incubated with polystyrene beads and cells adhering to or phagocytosing the beads were discounted. After thorough washing, PBL were incubated with either rhodamine-conjugated anti-human Ig (specific for γ, μ, α, κ, λ) or fluorescein-conjugated heat-aggregated human immunoglobulin, washed again thoroughly, and evaluated by alternate phase contrast and fluorescent microscopy for the percentage of positive cells.

E rosettes were prepared as described before[12], with minor modifications. Purified PBL and neuraminidase-treated SRBC were incubated together at 37° C, pelleted at 200*g*, and incubated at 4° C for a minimum of 30 min. The pellet was resuspended gently and counted by phase contrast microscopy. Lymphocytes were considered E-rosette-positive if SRBC were adherent. Approximately 90% of E rosettes involved three or more SRBC.

B and T cell markers were evaluated simultaneously on individual cells by first staining the PBL with the appropriate fluorescent reagent, washing thoroughly and then applying the rosetting technique. Individual cells were then viewed alternately under phase contrast and fluorescent illumination and scored for the presence of one, both or neither marker.

Peripheral blood lymphocytes from nine normal donors (two of the nine subjects were studied twice) were evaluated for percentages of B (surface Ig-bearing, aggregated Ig-binding) and T (E-rosette-forming) cells. Tests for the three markers were performed both separately and simultaneously (surface Ig+E rosettes and aggregated Ig-binding+E rosettes) for each patient on the same day using the same reagents (Table 1). Using the markers separately there was good agreement between markers both for individual donors and the group as a whole. Addition of the percentages for E rosettes and either of the B cell markers accounted for nearly all the lymphocytes (96.7 to 97.7%, range 89.0 to 102.5%). The data agree well with others[8,13]. Individual donors ranged widely in their populations of B and T cells and the same donor occasionally varied from day to day.

When these B and T cell markers were evaluated simultaneously on individual cells, a small subpopulation (mean 3.6%, range 1.0 to 6.0%) of lymphocytes bearing both markers was observed (Fig. 1). Several observations suggested that the detection of both markers on the same cell was not an artefact. (1) Each individual marker gave equivalent results whether evaluated separately or by the simultaneous technique (that is percentage surface Ig-bearing cells (separate) ≃ percentage surface Ig-bearing cells plus double marker cells (simultaneous)). Thus the markers seemed not to interfere with each other, and this suggested that use of the simultaneous technique was valid. (2) Almost all (>95%) double marker cells were heavily rosetted and therefore apparently had at least as high an affinity for SRBC as the usual T cell. (3) It seemed unlikely that the double marker cells were monocytes since they did not adhere to nylon, did not phagocytose and met the morphological criteria of small lymphocytes. Furthermore, monocytes apparently do not bind SRBC under the conditions of the E rosette test[8,11,12]. (4) An equivalent number of cells bearing both B and T cell markers was observed whether the B cell marker was surface-Ig-bearing or aggregated Ig-binding. These two markers have been shown to be independent[5–7]. (5) The simultaneous technique using E rosettes formed with non-neuraminidase-treated SRBC (such rosettes

Table 1 Comparative Study of Separate versus Simultaneous B and T Lymphocyte Surface Markers on Normal Human Peripheral Blood Lymphocytes

	Separate				Simultaneous				
Subject	Surface Ig	Aggregated Ig binding	E rosettes	Totals *	B cell marker		E rosettes	Both markers	No markers
Di	39.5 †	38.0	55.0	93.0–94.5	Surface Ig	37.0	56.0	5.0	2.0
He	34.5	32.5	64.0	96.5–98.5	Surface Ig	25.0	69.5	3.5	2.0
Co No. 1	28.0	27.5	71.0	98.5–99.0	Surface Ig	23.0	68.0	4.5	5.0
					Aggregated Ig binding	23.0	70.0	3.0	4.0
Co No. 2	25.5	26.0	76.0	101.5–102.0	Surface Ig	20.0	69.0	6.0	5.0
					Aggregated Ig binding	20.0	71.0	4.0	5.0
Br	22.5	20.0	79.0	99.0–101.5	Surface Ig	15.0	75.0	6.0	4.0
Bu	20.0	21.5	81.0	101.0–102.5	Surface Ig	17.0	79.0	2.0	2.0
					Aggregated Ig binding	16.0	77.0	3.0	4.0
Ho	15.0	12.0	77.0	89.0–92.0	Surface Ig	10.0	72.0	5.0	13.0
Ad No. 1	13.7	12.0	84.5	96.5–98.2	Surface Ig	9.0	81.0	5.0	5.0
					Aggregated Ig binding	9.0	82.0	4.0	5.0
Ad No. 2	6.3	6.8	91.0	97.3–97.8	Surface Ig	5.0	82.5	2.0	10.5
Fi	9.0	8.5	84.3	92.5–93.0	Surface Ig	6.0	88.0	2.0	4.0
Ev	7.7	7.0	90.0	97.0–97.7	Surface Ig	7.0	87.5	1.5	4.0
					Aggregated Ig binding	7.0	86.0	1.0	6.0
Range	6.3–39.5	6.8–38.0	55.0–91.0	89.0–102.5		5.0–37.0	56.0–88.0	1.0–6.0	2.0–13.0
Mean	20.2	19.2	77.5	96.7–97.7		15.6	75.8	3.6	5.0

* Totals obtained by addition of percentages for B and T cell markers.

† Numbers represent percentage of cells positive for each marker. Minimum 200 cells counted per experiment.

Table 2 Separate and Simultaneous B and T Lymphocyte Surface Marker Studies on "Clonal " Lymphocyte Populations

Lymphocytes	Separate Surface Ig	 Aggregated Ig binding	 E rosettes	Totals†	Simultaneous B cell marker		 E rosettes	 Both markers	 No markers
CLL‡ La	91.5*	90.5	9.0	99.5–100.5	Surface Ig	89.2	9.4	0.2	1.2
					Aggregated Ig binding	88.0	10.0	0.4	2.0
CLL Bi	97.5	96.5	3.0	99.5–100.5	Surface Ig	95.0	4.5	<0.2	0.5
					Aggregated Ig binding	96.3	3.0	<0.3	0.7
CLL St	0.2	0.5	94.5	94.7–95.0	Surface Ig	0.3	93.0	<0.3	6.7
					Aggregated Ig binding	0.3	95.0	<0.3	4.7
Lymphoblastoid line IM-1	97.0	96.5	<0.2	96.5–97.0	Surface Ig	98.0	<0.3	<0.3	2.0
					Aggregated Ig binding	98.5	<0.3	<0.3	1.5

* Numbers represent percentage of cells positive for each marker. Minimum 300 cells counted per experiment.
† Totals obtained by addition of percentages for B and T cell markers.
‡ CLL; chronic lymphocytic leukaemia.

Table 3 Effect of PHA Stimulation of Normal Human Peripheral Blood Lymphocytes on B and T Cell Surface Markers

Condition	Separate Surface Ig	 Aggregated Ig binding	 E rosettes	Totals†	Simultaneous B cell marker		 E rosettes	 Both markers	 No markers
Pre-culture	13.0*	12.5	81.0	93.5–94.0	Surface Ig	11.0	75.0	2.0	12.0
					Aggregated Ig binding	11.0	77.0	2.0	10.0
PBS	11.0	10.0	82.0	92.0–93.0	Surface Ig	9.0	78.5	1.5	11.0
					Aggregated Ig binding	9.5	78.0	1.0	11.5
PHA‡	10.5	11.0	83.0	93.5–94.0	Surface Ig	8.5	79.5	2.0	10.0
					Aggregated Ig binding	9.5	81.0	1.5	8.0

* Numbers represent percentage of cells positive for each marker. Minimum 200 cells counted per experiment.
† Totals obtained by addition of percentages for B and T cell markers.
‡ PHA concentration, culture conditions and length of culture were optimised to achieve maximal blastogenic response for the donor.

were more fragile and required longer contact at 4° C between PBL and SRBC) showed the same number of double marker cells. Thus, neuraminidase treatment of SRBC was not responsible for the double marker cells. (6) Double marker cells were not due to anti-SRBC antibodies. Several of the normal subjects (He, Co, Bu) were tested and had no such antibodies by haemagglutination. Furthermore, one patient with infectious mononucleosis and high titre anti-SRBC antibodies showed a percentage of double-marker cells (1.5%) comparable with normal donors. Also, the anti-Ig used in the simultaneous technique would be expected to inhibit SRBC binding by such antibodies. (7) It seemed unlikely that the double marker lymphocytes represented spontaneous antigen binding cells since the latter represent 0.14% or less of the nucleated cells in normal mouse blood[14]. (8) The simultaneous technique applied to lymphocytes which had not been passed over nylon fibre showed the same number of double marker cells. Thus lymphocyte contact with nylon was not responsible for the double marker cells.

It was reasoned that if double marker cells were produced by technical factors, then any lymphocyte population studied by the simultaneous technique would show such cells. Conversely, if cells bearing both B and T cell markers were a real subpopulation of PBL, then "clonal" lymphocyte populations should show few or no such double marker cells. One lymphoblastoid cell line and three patients with chronic lymphocytic leukaemia (CLL) were evaluated. The three CLL patients had white blood cell counts >50,000^{-3}, and in two cases the cells were of B cell origin and in one case apparently of T cell origin (Table 2). Double marker cells were virtually absent in these populations, thus supporting the view that cells bearing both B and T cell markers are a real subpopulation of PBL.

A markedly increased number of double marker cells (20.0% of PBL) has been encountered in a patient with hypogammaglobulinaemia (manuscript submitted). The meaning of this observation remains to be elucidated.

Attempts to remove (by trypsin digestion) and then regenerate surface Ig in normal PBL were unsuccessful and, therefore, direct evaluation of whether the double marker population represents T cells with the ability to adsorb Ig was not possible. As an alternative approach, normal PBL were evaluated for cells bearing both B and T cell markers both before and after stimulation by phytohaemagglutinin (PHA) in the presence of autologous serum (Table 3). PHA-stimulated PBL gave results equivalent to the controls, that is no increase in double marker cells was noted. Thus, PHA-

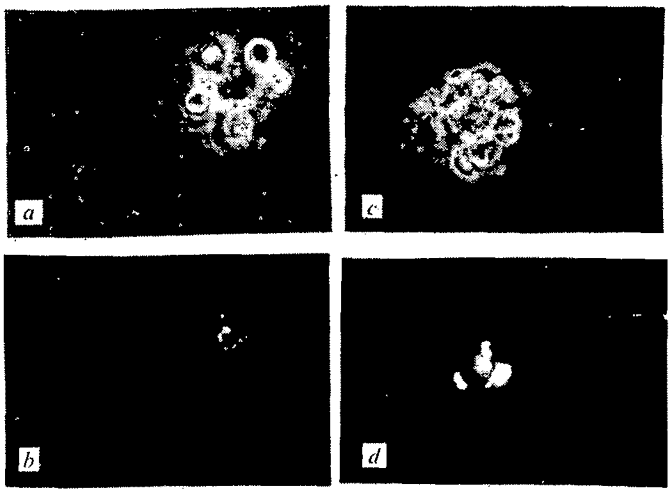

Fig. 1 Demonstration of normal human peripheral blood lymphocytes bearing both B and T cell markers. *a*, Phase contrast illumination of E-rosetted lymphocyte which under fluorescent illumination; *b*, reveals staining with rhodamine-conjugated anti-human Ig. Ig staining is clumpy because of incubation at 37° C during the E-rosette technique. *c*, Phase contrast illumination of E-rosetted lymphocyte which under fluorescent illumination; *d*, reveals binding of fluorescein-conjugated aggregated Ig. (×900.)

stimulated human PBL did not seem to adsorb Ig. Nevertheless, this does not rule out the possibility that *in vivo* stimulated or antigen-stimulated T cells possess this property.

Lymphocytes bearing neither B nor T cell markers ('null') were also found by the simultaneous technique (mean 5.0%, range 2.0 to 13.0%). This observation must be interpreted cautiously because of the fragility of E rosettes; however, 'null' cells may also represent a real subpopulation, as suggested by studies with the mouse[15].

Other investigators failed to detect lymphocytes bearing both B and T cell markers[8,9,13], but our study differed in several respects (methods of lymphocyte purification, specificity of antisera, intensity of fluorescent illumination, methods of preparation of E rosettes) which may account for this discrepancy. On the other hand, Bentwich *et al.*[11] reported double marker cells among the PBL of normal donors.

Some murine lymphoma lines have been reported which bear theta antigen, surface Ig and a receptor for Ig[16,17]. Such tumours could represent neoplastic transformation of a lymphocyte type normally present, and thus provide indirect evidence for the existence of a population bearing both B and T cell markers.

The nature of cells bearing both B and T cell markers remains unclear. Recent reports have presented evidence that some T lymphocytes can adsorb cytophilic antibody[18], and that antigen-activated T cells can bind antigen-antibody complexes[19] and bear easily detectable surface Ig[20]. If double marker cells prove to be activated T cells, the simultaneous technique should provide a method for identifying and isolating such cells. Alternatively, double marker cells may represent a subpopulation of B lymphocytes which can form E rosettes. Bentwich *et al.* reported that after neuraminidase treatment of PBL, a subpopulation of lymphocytes bearing surface Ig and the Fc receptor will form E rosettes[21]. However, electron microscopy has shown that such binding is different from the binding of SRBC to untreated PBL[11,21]. Thus, this observation may not be relevant to the data reported here. The third and most interesting possibility is that double marker cells may be able to function as B and (or) T lymphocytes, either as multipotential effector cells, or by further differentiation.

The study reported here provides evidence for the existence of a real subpopulation of lymphocytes bearing both B and T cell markers. Such evidence makes it necessary to reconsider the concept that all lymphocytes belong to one or the other of two distinct classes.

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Autoradiographic Visualisation of Paternal Chromosomes in Mouse Eggs

THE paternal component of the genome has been localised in the nuclear chromatin of early cleaving mouse eggs[1] by autoradiography following the mating of normal females with males in which the spermatogonial cell line was labelled *in vivo* with tritiated thymidine (^{3}H-TdR). The results indicated that in at least the earliest developmental stages of the mouse zygote there is a separation of maternal and paternal chromatin. This extends to mammals the concept of gonomery well known in lower animals[2]. The interpretation of these findings, as well as further exploitation of this experimental system, relies on the distribution of paternal labelled chromosomes at metaphase in early cleavage. An expected separation into twenty labelled and twenty unlabelled chromosomes is most unlikely to occur by chance. Here we present an analysis of the distribution pattern of the labelling in chromosomes.

Mice of the inbred CBA/H-T6/T6 strain were used. Intratesticular injections of ^{3}H-TdR in males, mating with superovulated females, checking of the days of peak production of labelled sperm, collection of zygotes in the oviducts, spreading by air-drying[3], preparation and staining of autoradiographs, were performed as described previously[1]. The time of collection of the zygotes, calculated from the time of the appearance of the vaginal plug, however, was selected for obtaining eggs close to or at the four cell stage (60 h post-mating). In addition, well-spread metaphases of cleaving eggs were obtained by injecting colchicine directly into the uterine lumen 2 to 4 h before killing the pregnant mice. It must also be pointed out that the intratesticular injection of ^{3}H-TdR results in continuous availability of the tracer for at least 2 d (ref. 4).

Out of 428 well-fixed eggs from 264 females examined, 129 were in metaphase. Of these, forty-one contained twenty labelled chromosomes and eighty-eight had grains in less than twenty chromosomes. Particularly well-spread metaphases allowed arrangement into karyotypes (Fig. 1) in which the paired assortment of homologous chromosomes was established, including the sexual pair and the small translocated T6 marker. The number of labelled chromosomes never exceeded twenty in any metaphases observed, thus giving an autoradiographic visualisation of the classical laws of Van Beneden and Boveri[2], namely that the number of chromosomes contributed by each parental pronucleus to the diploid zygote is haploid. Labelling of only part of the presumed paternal component, which varied from one to nineteen labelled chromosomes, is probably due to fertilisation by sperm derived from spermatogonia which were exposed to ^{3}H-TdR only during the late part of DNA synthesis. In fact, approximately one-half of the partially labelled metaphases had a labelled chromosome morphologically identifiable as a late-

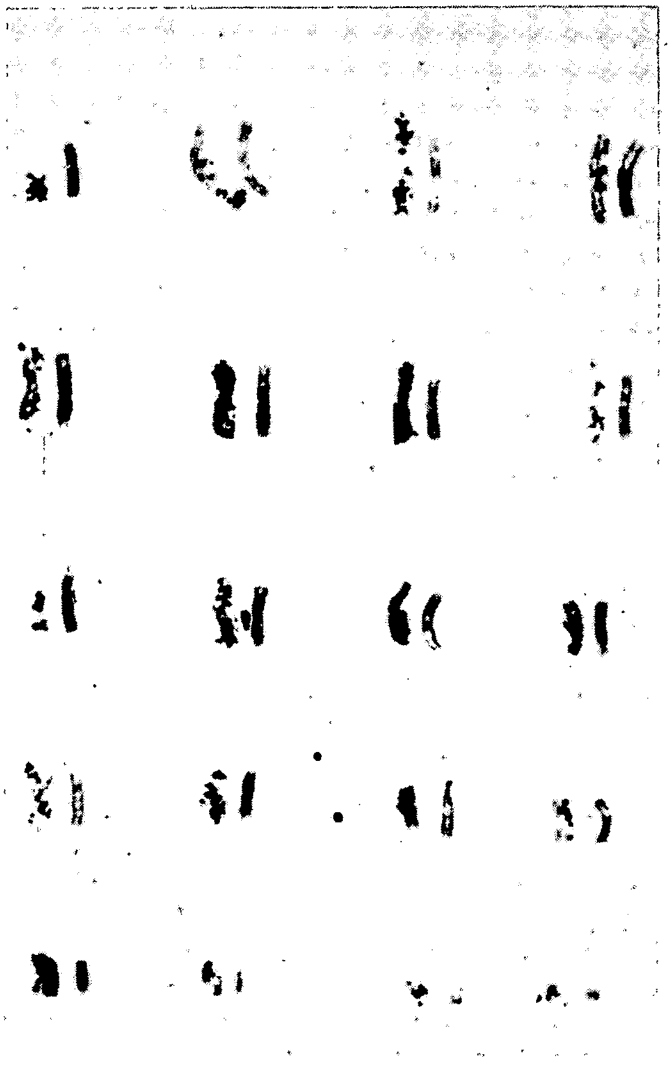

Fig. 1 Karyotype of a metaphase in a mouse egg at the second division of cleavage. The paternal chromosome in each homologous pair is labelled, the maternal is not, including the XY sexual pair (top left) and the T6 marker (bottom right).

labelling Y chromosome. Moreover, only the Y was labelled in all male metaphases in which only one chromosome contained grains. This is consistent with the late replication of DNA by the Y chromosome before meiosis in male mice[4]. Labelling of autosomes in the partly labelled metaphases varied markedly both in the localisation of grains over different parts of the chromosomes and in the number of labelled chromosomes. The labelling pattern generally corresponded to morphological distinctions previously made on meiotic autosomal bivalents[4], based on the observation that the late replicating parts of the genome are heterochromatic.

It cannot be excluded that tritium-induced lesions cause morphological anomalies. Even if these may have been missed because of the previous selection of morphologically well-preserved eggs in which extensive exchanges between homologous chromosome pairs had not occurred, the observed labelling pattern cannot have been generated by radiobiological effects.

It must also be emphasised that the relative proximity of labelled or unlabelled chromosomes on spread metaphase preparations does not necessarily reflect their relative position in the living unfixed egg. Indeed, phase contrast microscopic examination of the eggs during the fixation and spreading revealed distortion of the original interchromosomal arrangement.

These results indicate that an expected distribution of labelled chromosomes on metaphase plates at subsequent mitoses can be observed in eggs fertilised by ^{3}H-TdR-labelled sperm.

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Allelic Contracomplementation Possible Mechanism for Initiating Sympatric Speciation

IN *Drosophila melanogaster*, certain heterozygous combinations of viable alleles of the sex-linked Abruptex locus are lethal. This new type of allelic interaction has been called contracomplementation[1]. The phenomenon is equivalent or related to negative complementation, but the term contracomplementation is more descriptive in this specific case. The following heterozygous combinations of viable Abruptex alleles are found to be lethal: Ax^{9B2}/Ax^{E2}, Ax^{9B2}/Ax^{16172} (ref. 2), Ax^{28}/Ax^{71d} (ref. 1), Ax^{71d}/Ax^{9B2}, and Ax^{28}/Ax^{16172}. Interallelic complementation has been supposed to have certain evolutionary significance; it is an intermediate stage in the evolution of heteromultimeric proteins, such as human haemoglobin, from homomultimeric proteins[3]. Thus, interallelic complementation may be a stage in the progressive evolution of a population. Contracomplementation as an opposite of complementation should accordingly have an 'opposite' evolutionary significance, being an initiation mechanism in splitting one population into two parts, that is an initiation mechanism of sympatric speciation.

Sex-linked genes of different laboratory stocks of *D. melanogaster* which carry contracomplementing alleles of the *Ax*-locus never recombine (except by laboratory manipulations). Supposing that contracomplementing alleles are in the autosomes, stocks carrying such alleles are conceptually different species because they are reproductively isolated from each other. If we presume that a uniform natural population consists of a^+/a^+ individuals and two contracomplementing mutations, a^x and a^y, occur with a certain rate in this population, the frequency of the mutant allelles will increase if both mutations are favoured by selection (a^x/a^x, a^x/a^+, a^y/a^y, and a^y/a^+ individuals have greater fitness than a^+/a^+ individuals). If, because of contracomplementation, a^x/a^y individuals are lethal or otherwise less fit than other genotypes, the population will virtually split into parts, one containing a^x alleles and the other a^y alleles because genes prohibiting mating between individuals carrying a^x and a^y alleles become beneficial. It is thus suggested that contracomplementation is a possible initiation mechanism of sympatric speciation but the allelic interaction process itself should be studied more profoundly.

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No Mouse PMN Leukocyte Depression after Inoculation with Brain Tissue from Multiple Sclerosis or Spongiform Encephalopathies

RECENT experiments by Carp *et al.*[1,2] and Licursi *et al.*[3] have excited hopes among investigators of multiple sclerosis (MS) and slow virus infections of the central nervous system that an experimental marker for these diseases has finally been found. Tissues from humans with MS or from scrapie mice were inoculated into normal mice, which showed a polymorphonuclear leukocyte (PMN) depression beginning within 1 to 3 d of the inoculation and persisting many months thereafter.

We report here our failure to confirm these results for MS and scrapie or to obtain a PMN depression with the human subacute spongiform virus encephalopathies, kuru and Creutzfeldt–Jakob (C–J) disease.

C57Bl/6J mice were obtained from the Jackson Laboratories, Bar Harbor, Maine. They were distributed into test groups of not more than six mice per cage and held for 2 weeks before experiments were begun. No fighting was observed. No antibiotics were added to their diet of water and mouse chow.

Scrapie mouse brain was used from tenth mouse brain passage of the Compton strain, originally isolated from sheep and previously passaged nine times in goats. Normal mouse brain was prepared from healthy adult NIH Swiss–Webster mice. Kuru chimpanzee brain was obtained from chimpanzee A191, inoculated with fourth passage chimpanzee brain material from the Eiru strain. This animal showed the typical clinical and pathological features of kuru, and the inoculum used in the present experiment has transmitted kuru in subsequent chimpanzee passage. C–J chimpanzee brain was obtained from chimpanzee A122, inoculated with first passage chimpanzee brain material from the Woolfe strain. This animal showed the typical clinical and pathological features of C–J disease, but its tissues have not been re-inoculated into primates. Normal chimpanzee brain was obtained from an uninoculated healthy young adult animal. Each of the above brain tissues was ground in a mortar and pestle using sterile sand, diluted to a 10% (w/v) suspension in phosphate-buffered saline, *p*H 7.4, centrifuged for 20 min at 2,000 r.p.m., and the supernatant fluid stored at −70° C until use. Human MS brain was prepared from autopsy material obtained from patient L. K. who had had a characteristic relapsing illness for 5 yr and had multifocal plaques throughout the white matter of the brain. A coronal section was dissected into grey and white matter, the tissues separately homogenised in an ice-cooled Sorvall blender for a total of 1 min, diluted to a 10% (w/v) suspension in Eagle's Basal Medium, and after centrifugation for 20 min at 2,000 r.p.m. the supernatant fluid was stored at −70° C until use. In addition, Dr Richard Carp provided coded aliquots of a pooled suspension of 10% (w/v) normal human brain and MS human brain. Details of their preparation are described elsewhere[1].

All inoculated mice received 0.03 ml volumes of the dilutions intracerebrally. For leukocyte measurements, blood was drawn into capillary pipettes from which a drop was released for the immediate preparation of slides. 20 μl of the remaining blood was quickly mixed with 10 ml of Isoton in plastic cuvettes to which 3 drops of Zap-Isoton was added, and the white blood cell (WBC) counts measured in a Model B Coulter Counter. The Wright's stained slides were coded and a 200 cell differential count was performed to obtain the percentage of PMN cells. The code was broken only at the end of each experiment.

Results of the PMN counts in each of three experiments are shown in Table 1.

In experiment one, groups of ten mice were inoculated with each tissue homogenate, and each inoculum group was separately caged. Individual mice in each cage were identified by an ear snip pattern. Mice were bled retro-orbitally without anaesthesia, and each mouse was bled before inoculation and weekly thereafter for 4 weeks.

Instead of a depression there was an elevation of the PMN count in all groups, and the differences between groups were not significant. If only those counts lower than 20% are analysed, the total number of post-inoculation determinations is reduced from 200 to 129, and the average PMN counts to between 12 and 15%—again with no significant differences between the groups. WBC counts were also elevated from pre-inoculation averages between 6,500 and 9,000 cells mm^{-3} to levels from 10,000 to 13,500, with a tendency for entire cages of mice to show comparable elevations in any given week. The mice inoculated with normal mouse brain and those inoculated with scrapie mouse brain were held several more months. All scrapie mice died between 5 and 7 months following inoculation. All normal mice remained well.

Table 1 Percentage of PMN in C57Bl/6J Mouse Blood Before and After Intracerebral Inoculation of Brain Tissue Extracts

Inoculation group	0	Weeks after inoculation 1	2	3	4	Post-inoculation average
Experiment 1						
Normal mouse	12	15	12	24	24	19
Scrapie mouse	9	15	17	17	19	17
Normal chimp	8	16	13	21	14	16
Kuru chimp	9	15	14	16	17	16
C-J chimp	10	26	22	19	24	23
Experiment 2						
Uninoculated	10	16	13	11	11	13
MS (white)	10	11	15	—	15	13
MS (grey)	10	11	14	12*	13†	13
MS pool	10	13	8*	13†	12	11
Normal pool	10	12	16	12*	14	13
Experiment 3						
Uninoculated	14	13	8	9	9	10
Scrapie mouse	14	12	—	13	9	11
MS pool	14	13	11	10	8	10
Normal pool	14	16	8	8	10‡	10

All percentages are based on 200 cell differential counts of Wright's stained blood smears. There are ten mice per experimental point in experiment 1, four mice per point in experiment 2 and five mice per point in experiment 3.

* 3 mice in group.
† 2 mice in group.
‡ 4 mice in group.

In experiment two, sixteen cages containing six mice per cage were handled as follows. One mouse from each cage was bled for control counts on the day of inoculation. The remaining five mice were identified by an ear snip pattern, one was inoculated with each of the four tissue homogenates and the fifth mouse left uninoculated. Four cages of mice were bled and then discarded each week for 4 weeks (each mouse was thus bled only once) giving four mice per experimental point. Mice were bled retro-orbitally without anaesthesia.

From a pre-inoculation average PMN count of 10%, slight average elevations occurred which were not significantly different one from another. The single exception was a depression during the second week in the group of mice inoculated with the pooled human MS brain. This depression was no longer evident in the following 2 weeks, and the combined 4 week values were not significantly different between groups. WBC counts remained in the 5,000 to 10,000 range throughout the experiment.

In experiment three, sixteen cages containing six mice per cage were handled as in experiment two, except that, following the control bleeding, all mice in each of four cages were inoculated with a given tissue homogenate, and four cages of mice were left uninoculated. Each week for 4 weeks one cage of five mice having received one of the homogenates and one cage of uninoculated mice were bled and discarded. Mice were anaesthetised with ether for 30 s and bled from the exposed axillary vessels.

From a pre-inoculation average of 14%, the PMN counts tended to fall slightly, but in no case was the depression significantly different between the inoculated and uninoculated groups of mice. (One cage of scrapie-inoculated mice had PMN counts between 30 and 75% 2 weeks after inoculation and are omitted from the table.) The combined 4 week values were virtually identical, and not significantly lower than the pre-inoculation average. WBC counts remained in the 4,000 to 9,000 range throughout the experiment.

WBC and PMN counts from the first experiment suggested that a leukocyte stimulus was operating in all groups of mice irrespective of the inoculum given. Cage infections appeared the most likely cause, but sporadic eye infections, anxiety of the unanaesthetised mice, and repeated bleedings of the same mice may also have contributed to the results. Whatever the stimulus, it was uninfluenced by inoculation of scrapie, kuru, or C–J disease-infected brain compared with normal brain. This was equally true when instead of all the data, 'high' PMN counts ($\geq 20\%$) were excluded from analysis. Thus it could be said that if a PMN depressing factor was in fact produced by the infected brain homogenates, it was totally overcome by exogenous leukocyte stimulation.

In the second experiment, by using each mouse only once, randomising the mice within cages, and including uninoculated controls, we were able to avoid the leukocyte stimulus seen in the first experiment. We also included inocula given by Dr R. I. Carp from MS and normal brain tissue that had produced in his laboratory definite PMN depression (MS) and no PMN depression (normal). We found no effect from either these or our own inocula.

A final experiment was then done in which mice were anaesthetised and bled from the axillary vessels, duplicating the technique used by Carp. We also used new aliquots from his MS and normal brain pools. The scrapie material used, as also in our first experiment, was that which we had originally given to Carp for his experiments[2,3]. Once again, we were unable to distinguish between any of the inoculated groups and the uninoculated group, although there was an overall trend to slightly lowered PMN counts during the 4 week period of study.

We must thus conclude that in spite of efforts to duplicate exactly the protocol of the Staten Island group, using the same strain of mice purchased from the same source, some of the same inocula, and the same methods (including the bleeding technique), we cannot confirm that either scrapie or MS brain tissue inoculated into mice depresses the PMN count, nor have we seen any effect in mice inoculated with brain from kuru or C–J disease.

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Sensitivity of Human Foetal Intestine to Interferon

ACUTE infectious non-bacterial gastroenteritis is a common disease in which recovery usually occurs within 24–48 h of the onset of diarrhoea and vomiting[1,2]. This short duration suggests that a mechanism of rapid onset, such as interferon, may play a significant role in recovery. The role of interferon in recovery from acute enteric viral infections of man is, however, unknown. Indeed, little is known about the ability of the human intestine to either produce or respond to interferon. We have therefore investigated the role of interferon in viral infections of the human gut. We found that human foetal intestine is quite responsive to the antiviral properties of exogenous interferon, and when so treated exhibits a marked inhibition of the replication of an enteric virus, echovirus type 11.

Previous studies of interferon in intestinal secretions have yielded little information because of the rapid proteolytic enzymatic inactivation of interferon, in spite of the addition of trypsin inhibitors[3]. We have also found that interferon is inactivated completely when added to human duodenal secretions at a final concentration of 500 U ml^{-1}, even when the secretions first have soybean trypsin inhibitor type II-S added at a concentration of 0.67% or foetal calf serum at a concentration of 16%.

A new technique for maintaining fragments of human foetal small intestine in organ culture for up to 3 weeks[4] presents a unique opportunity to study the role of interferon in human intestine, free from proteolytic enzymes. Unlike monolayer cell cultures, organ cultures maintain the *in vivo* anatomic relationships as well as certain physiological functions of the intestine[4]. Human foetal intestinal organ cultures were prepared and maintained as previously described[4]. Human interferon was prepared in human leukocytes stimulated by ultraviolet irradiated Sendai virus, using the method of Cantell[5], and was provided by Drs S. Baron and C. Buckler.

Figure 1 summarises the procedure and results of an experiment in which organ cultures were pretreated for 18 h with exogeneous human interferon and then challenged with echovirus 11. In the absence of interferon, echovirus 11 replicated to titres comparable with those reported in intestinal organ cultures[6]. By contrast, cultures pretreated for 18 h with 5 U ml^{-1} (reference units, see below) or more of interferon failed to support virus replication. Interferon pretreatment with less than 5 U ml^{-1} permitted virus growth. It is impossible at present to study in this way the replication of aetiological agents of acute infectious non-bacterial gastroenteritis, such as the Norwalk agent[2], because there is no assay system available except human infectivity[2].

Having demonstrated the sensitivity of human foetal intestinal organ cultures to interferon, we attempted to determine whether these cultures could be stimulated to synthesise interferon. Several potential inducing agents were used, including polyriboinosinic polyribocytidylic acid[7] (poly(I)·poly(C), Microbiological Associates, Bethesda, Maryland), echovirus type 11, and the Norwalk agent from a filtrate known to be infectious for man. Several other techniques which augment interferon production were also used; these included interferon priming[8], diethylaminoethyl (DEAE) dextran[9,10] (Pharmacia), and treatment after induction with cycloheximide[11,12] (Sigma). Table 1 summarises

Table 1 Attempts to Stimulate Interferon Production by Human Foetal Intestinal Organ Cultures

Experiment	Priming with human interferon 10 U ml^{-1}		Inducing agent*			Post-induction augmentation	Interferon assay
	5 h incubation at 36° C	18 h incubation at 36° C	Poly (I) · poly (C) 50 μg ml^{-1} + DEAE-dextran 100 μg ml^{-1} for 90 min	Echovirus 11, 1,000 TCID$_{50}$	Norwalk agent 0.3 ml of 1 : 10 dilution	Cycloheximide 10 μg ml^{-1} for 3 h	Media/explants
1	−	−	+	−	−	−	Negative/negative
2	+ †	−	+	−	−	−	Negative/not done
3	−	+	+	−	−	−	Negative/negative
4	−	+	+	−	−	+	Negative/negative
5	−	−	+	−	−	+	Negative/negative
6	−	−	−	+ ‡	−	−	Negative/negative
7	+	−	−	+	−	−	Negative/negative
8	−	−	−	−	+	−	Negative/negative
9	+	−	−	−	+	−	Negative/negative

For priming, interferon was added in 1.25 ml of media to cultures, incubated as indicated, and cultures were then washed three times with fresh media and exposed to inducing agent.

For poly(I)·poly(C) and DEAE-dextran induction, poly(I)·poly(C) of a lot known to induce interferon in human leukocytes and DEAE-dextran were added in 1.25 ml of media to cultures as indicated. Plates were then washed three times, renewed with media, incubated at 36° C for 20 h, and fluids were then collected, as were explants, and frozen at −80° C until assayed for interferon. In experiments 4 and 5, cultures were incubated as indicated with cycloheximide, after removal of poly(I)·poly(C)-dextran, then washed three times, renewed with media, incubated for 20 h, and fluids and explants were then collected.

For virus inoculation, echovirus 11 or Norwalk agent (8FIIa filtrate) was added in 1.5 ml of media to cultures as indicated. Plates were then incubated at 36° C for 20 h, and fluids were collected, as were explants, and frozen at −80° C. Explants from duplicate plates were freeze-thawed three times in 1.5 ml media, and homogenised in a glass tissue homogeniser. Clarified fluid collections and tissue homogenates were ultraviolet-irradiated for 8 min at 2.5 inches from an Atlantic germicidal 282L-20 ultraviolet light bulb, an intensity shown to inactivate completely the infectivity of 10^6 TCID$_{50}$ of echovirus 11.

Interferon assay was performed on undiluted and 1 : 5 diluted media and explant homogenate collections, using the VSV cytopathogenicity inhibition assay in human foreskin fibroblast cells.

* Induction was also attempted using 100, 50, 10 and 1 μg ml^{-1} of poly(I)·poly(C) alone for 1 and 18 h.

† This experiment was also performed using 100 U ml^{-1} of interferon.

‡ This experiment was also performed using 10^6 TCID$_{50}$ echovirus 11.

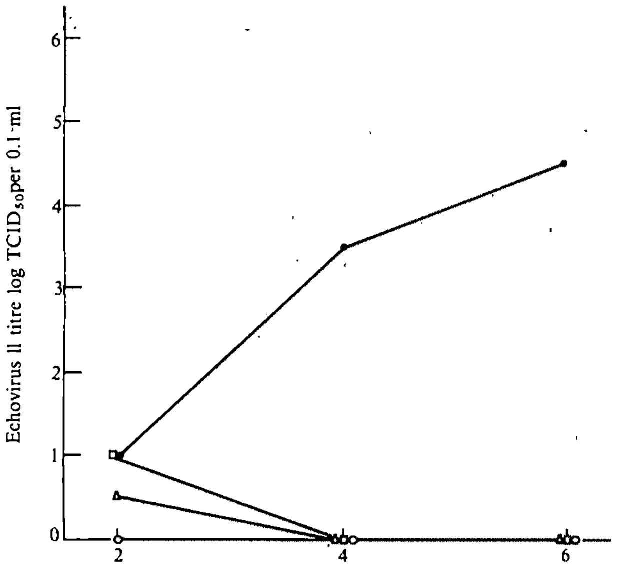

Fig. 1 Effect of exogenous human interferon on echovirus type 11 replication in human foetal small intestinal organ cultures. Organ cultures were prepared from foetal intestine 12–18 weeks old, and maintained for 5 d at 36° C, with the media changed every other day before the start of experiment. Cultures were pretreated with the indicated doses of human interferon in 1.25 ml media for 18 h and then washed three times with fresh media. Echovirus 11, 100 TCID$_{50}$, was then inoculated per culture dish for 4 h at 36° C; cultures were then washed three times with fresh media, renewed with appropriate interferon-containing media, and incubated. Fluids were collected and cultures refed with appropriate interferon-containing media on days 2, 4, and 6 after virus inoculation. All experiments were run on duplicate plates with pooling of collected fluids. Assays for echovirus in collected fluids were performed in WI-38 cells. ●, Control; ○, 20 U ml^{-1} interferon; □, 10 U ml^{-1} interferon; △, 5 U ml^{-1} interferon.

the procedure and results. In no case could we demonstrate interferon synthesis by human foetal intestinal organ cultures using the VSV cytopathogenicity inhibition assay[13]. These results were obtained in assays in which known positive control preparations of 5 U ml^{-1} of human leukocyte interferon could be detected. The standard reference human interferon 6,787 titres 100 U ml^{-1} by this method and the standard reference human interferon 6,919 titres 5,000 U ml^{-1} by this method.

Our demonstration of the sensitivity of human foetal intestine to exogenous interferon was made possible by a newly developed intestinal organ culture technique. This technique has obviated in part the experimental difficulties in studying the role of interferon in the gastrointestinal tract posed by proteolytic enzymes in intestinal secretions. Indeed, because of the rapid inactivation of interferon in intestinal secretions, it is doubtful whether intraluminal interferon plays any role in the recovery from viral infections of the gastrointestinal tract. Rather, it is possible that interferon may spread cell-to-cell within the intestinal mucosa, as has been suggested in other tissues[14].

The inability to demonstrate synthesis of interferon by human foetal intestinal organ cultures does not necessarily imply that adult intestine *in vivo* cannot produce interferon. On the other hand, it may be that while the intestine is sensitive to the effects of interferon, cells normally present in adult intestine are unable to produce interferon. In this regard, we have recently demonstrated that one of the most significant pathological features during acute illness with the Norwalk agent of non-bacterial gastroenteritis is a marked round cell infiltration in the lamina propria of the proximal small intestine[15]. It is known that lymphocytes possess the ability to synthesise interferon[16,17]. Conceivably, the round cell infiltrate induced by the Norwalk agent could synthesise local interferon, which in turn could protect the intestine from further viral replication and account for the rapid recovery usually seen in acute infectious non-bacterial gastroenteritis.

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Hydroxyapatite formed from Coral Skeletal Carbonate by Hydrothermal Exchange

WE have prepared porous microstructures, having potential for prosthetic applications, consisting of hydroxapatite and of whitlockite, by employing exchange reactions at elevated temperatures and pressures. Rather demanding specifications are to be met by biomaterials being considered for tooth or bone replacement. Porous solids have the advantage of allowing circulation of body fluids and of increasing the potential for firm attachment of body tissue[1,2]. Weber *et al.*[3] successfully replicated the porous structure of echinoid skeletal material in epoxy resin and in sodium silicate; and White *et al.*[4] achieved replication of the coral *Porites* structure with methacrylate, tin, Tichonium, and sintered Al_2O_3. This was the starting point for our attempts to reproduce such structures in the phosphatic material which is a major component of human teeth and bones, hydroxyapatite. Such a material, porous, inorganic and sterile (formed at high temperatures and pressures), should be highly compatible with body tissue, and when used as bone implants may become essentially integral with the bone. Furthermore, composites of such porous hydroxyapatite with other materials including metals[4] may achieve optimum mechanical and physical properties for a particular application.

We anticipated that by choice of suitable experimental parameters it would be possible to replace with phosphatic material the porous calcium carbonate skeletal structure of some marine invertebrates. Hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ is harder (Mohs hardness = 5) than either the aragonite (= 3.5–4) or calcite (= 3) forms of $CaCO_3$ and therefore such exchange has the potential to form structures which are stronger than the skeletal material of the original marine organism. It would, of course, have the advantage that it would be chemically and mineralogically 'identical' with normal human hard tissue, and would be stable in contact with body fluids, while the carbonate is not. High pressure and high temperature treatment would furthermore solve problems of sterilisation.

Slices of the massive scleractinian coral, *Porites*, and spines of the asteroid *Acanthaster planci* were used as starting materials to provide the aragonite and calcite polymorphs of $CaCO_3$, respectively. Hydrothermal techniques[5] were used, since it was expected that elevated temperatures and pressures would hasten any replacement process. The sections of *Porites* coral, or of the *Acanthaster planci* spine, together with weighed quantities of reactants and water were sealed in a gold tube, heated at specified temperatures and pressures for periods of time varying from 12 h to 1 week, cooled and the products examined.

Essentially complete replacement of aragonite by phosphatic material has been achieved under particular conditions. The porous interconnecting structures were preserved, as shown in the scanning electron micrographs of Fig. 1*a* and *b*. Figure 1*a* shows the material which is hydroxyapatite, having replaced the original *Porites* aragonite[4] and preserved its structure.

Typical experimental conditions for the exchange reaction to produce hydroxyapatite are given in Table 1, along with data on the asteroid material. Experiments resulting in replacement of the aragonite are listed and, for contrast, examples of less favourable conditions. We assume that under favourable conditions the following exchange reaction takes place.

$$\underset{\text{aragonite}}{10CaCO_3}+6(NH_4)_2HPO_4+2H_2O\rightarrow \underset{\text{OH-apatite}}{Ca_{10}(PO_4)_6(OH)_2}+6(NH_4)_2CO_3+4H_2CO_3$$

Observations under the polarising microscope are recorded as well; and phase identification was made by X-ray diffraction as well as observations under the microscope.

The *Acanthaster planci* spines were completely converted to whitlockite (β-$3CaO.P_2O_5$) as shown in Table 1 and Fig. 1*b*. For the formation of whitlockite, the previous reaction may be modified accordingly:

$$\underset{\text{calcite}}{3CaCO_3}+2(NH_4)_2HPO_4\rightarrow \underset{\text{whitlockite}}{2[Ca_3(PO_4)_2]}+2(NH_4)_2CO_3+2H_2CO_3$$

The microstructure of the whitlockite product is very close to that of the original carbonate skeletal material, shown by Weber *et al.*[3,4,6,7].

Some very tentative tensile strength data are given in Table 1, obtained on both the original coral aragonite, and on the hydroxyapatite product. The measurements were made on rectangular bars approximately 0.15 × 0.15 × 1 inch using the 4-point loading method. Whereas the strength measurements were not sufficient in number to be precisely determined, they indicate that the exchange products are generally as strong as or stronger than the original coral, and provide considerable encouragement for extension of the investigation.

The unit cell dimensions of two apatite samples were refined, using the thirteen strongest lines below $2\theta = 50.5°$ (Table 2).

There appears to be a real increase in cell volume, ~0.35%, over the reference values for CO_2-free OH-apatite crystallised at higher temperatures. The X-ray peaks are slightly broader than in hydroxyapatite crystallised hydrothermally at higher temperatures. Several effects may account for the cell increase: incorporation of CO_3^{2-}, a very small amount of Sr^{2+}, and possibly some NH_4^+, as well as the enlargement which is also observed in hydroxyapatites precipitated at low temperatures.

Semi-quantitative spectrographic analysis data for a typical

Table 1 Hydrothermal Phosphate Exchange of Coral (*Porites*) and Echinoderm Spine Material (*Acanthaster planci*)

Sample number	Reactant	Temperature (° C)	Pressure (pound inch^{-2})	Time (h)	Result (phase)	Optical or other observation	Microstructure	Strength* g cm^{-2}	Young's modulus g cm^{-2}
Coral									
4h	$(NH_4)_2HPO_4$	250	15,000	24	Apatite				
4g	$(NH_4)_2HPO_4$	260	15,000	12	Apatite				
4c	$(NH_4)_2HPO_4+Ca(OH)_2$	250	15,000	24	Apatite+l.Cc				
5e	$(NH_4)_2HPO_4+Ca(OH)_2$	260	15,000	48	Apatite	Spherulitic birefringence	Open pores replica of coral	3.1×10^4	5.2×10^6
5d	$(NH_4)_2HPO_4+Ca(OH)_2$	350	15,000	24	Apatite+Cc			2.2×10^4	5.0×10^6
5c	$(NH_4)_2HPO_4+Ca(OH)_2$	270	15,000	24	Apatite	Wavy, spherulitic		3.3×10^4	5.8×10^6
4i	$(NH_4)_2HPO_4$	260	15,000	24	Apatite+v.l. calcite	Wavy extinction, spherulitic		1.9×10^4	4.7×10^6
4l	$(NH_4)_2HPO_4$	180	15,000	24	Aragonite+ apatite				
4j	$(NH_4)_2HPO_4$	260	15,000	48	Apatite	(Partly leaked)	Open pores	2.5×10^4	6.0×10^6
Spine									
2c	$(NH_4)_2HPO_4$	260	15,000	24	Whitlockite	Epitaxy partial	Replica of original (partly filled in)		
2d†	$(NH_4)_2HPO_4$	260	8,000	24	Whitlockite	(Microstructure more filled in)			
1g†	$(NH_4)_2HPO_4+Ca(OH)_2$	260	15,000	24	Whitlockite +l. calcite		Replica of original (not as good as 2c)		

* Strength from four-point loading: original aragonite=2.84×10^4 g cm^{-2}, Young's modulus=5.75×10^6 g cm^{-2}.
† Sea urchin, *Diadema*, rather than *Acanthaster planci*.

apatite derived from coral (No. 4i), after washing in dilute acetic acid, are given in Table 3.

Table 2 Cell Dimensions of Apatite Samples

Sample	a_0	Co	Cell volume
4i	9.435±0.001	6.893±0.001	531.4
5c	9.441±0.002	6.888±0.003	531.7
(reference No. 19)	9.422	6.883	529.2
9, 10*	9.426±0.001	6.884±0.001	529.7
Holly Springs 9, 10*	9.426±0.001	6.884±0.001	529.7

* The two reference measurements made in our laboratory are of synthetic OH-apatite crystallised at 800° C, and of natural OH-apatite (containing impurities) from Holly Springs, Ga. (ref. 9).

Electron microprobe analyses for Ca : P ratio showed approximately the same value as for typical OH-apatites crystallised at higher temperatures, assumed to be stoichiometric.

Infrared absorption spectra taken of typical examples of the exchanged specimens reveal small absorption peaks due to the presence of CO_3^{2-} substituted in the hydroxyapatite structure. The CO_3^{2-} spectra appear to be explained as a composite of peaks at 884, 1,465, and 1,534 cm^{-1} and of peaks at 864, 1,430, and 1,455 cm^{-1}. This indicates that CO_3^{2-} substitutes both for OH^- in the columnar positions, and for tetrahedral PO_4^{3-} (ref. 8). Similar observations concerning infrared absorption spectra have been made by Eysel and Roy[9,10] in synthetic apatites produced in the same pressure-temperature range from chemical mixtures into which CO_2 has deliberately been introduced. These data suggest that there is a maximum concentration of CO_3^{2-} which may substitute for OH^- or PO_4^{3-} under a particular set of conditions.

The full explanation for the phenomenon observed, that hydroxyapatite was formed from aragonite-$CaCO_3$, while whitlockite was formed from calcite-$CaCO_3$ has not yet been found, and probably involves complexities of nucleation and crystal growth, as well as phase stability[11,12]. Indeed, two specimens of coral and spines sealed together in the same tube produced, respectively, hydroxyapatite and whitlockite. Calcite spines of *Acanthaster planci* are magnesium-rich[6], and magnesium is clearly associated with whitlockite: up to 17 atom % Mg has been found to substitute for Ca in whitlockite[13], while its solid solution in hydroxyapatite is very limited[17]. Naray-Szabo[14] pointed out that Mg^{2+} 'de-stabilises' the apatite lattice; examples are known of whitlockite (rather than hydroxyapatite) formation[15] in dental calculus in which there is positive correlation of whitlockite content with magnesium; and most whitlockite natural mineral deposits contain significant amounts of magnesium.

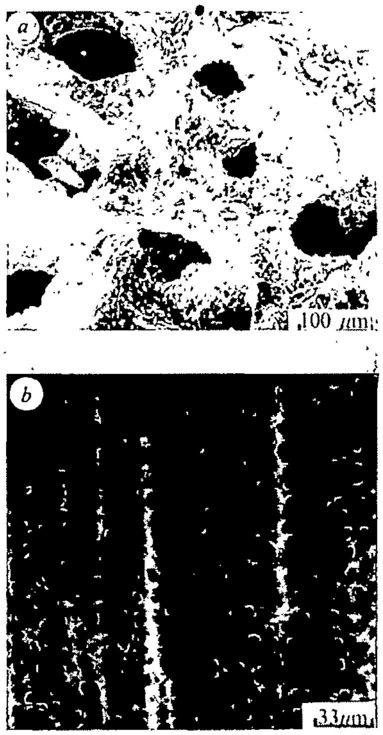

Fig. 1 *a*, Hydroxyapatite replacement of coral *Porites* structure; *b*, whitlockite replacement of *Acanthaster planci* spine, originally composed of high-magnesium calcite.

The major concern of the present work, however, is the exchange process by which the porous coral structure preserved as hydroxyapatite (containing some CO_3) is formed. There are

Table 3 Spectrographic Analysis of a Typical Apatite Derived from Coral

% Oxide	% Oxide
0.2	MgO
0.02	SiO_2
<0.007	Mn_2O_3
0.007	Fe_2O_3
<0.003	Y_2O_3
1.0	SrO
0.006	La_2O_3
<0.01	CeO_2
<0.01	Ce_2O_3
0.002	Al_2O_3
0.002	TiO_2
0.1	NiO
0.05	BaO
0.1	Na_2O

Not detected: Cr, Be, Mo, Cu, Ag, Yb, Zn, Cd, Bi, Sb, In, Ge, Ga, Pb, Th, Sc.

certain crystal structural similarities between aragonite and apatite, which obviously favour this morphological preservation[10].

The *Acanthaster* spines consist of single crystal calcite[7], which could result in ordered epitaxial growth of single crystal whitlockite. While the crystal structure of whitlockite is currently being worked out in detail (A. L. Mackay, reference in ref. 16; and refs 13, 17, 18), the fact that the external morphology is rhombohedral, similar to that of calcite, and its space group (R3c) is similar to that of calcite (R$\bar{3}$c), suggests the possibility of such structural control (D. M. Roy and D. Dinger, unpublished).

In conclusion, we have produced porous structures which have great potential for use in human implants, in the material which is the major inorganic component of tooth and bone, and which should be most readily accepted by the body, hydroxyapatite. The second phosphatic material produced, whitlockite, may also have potential for such applications. The hard tissue of the massive scleractinian coral, *Porites*, which was used as the starting material for producing the hydroxyapatite materials, exists in very large quantity, so there is essentially no limit on its availability. Furthermore, the conversion to hydroxyapatite was achieved at elevated temperatures and pressures, insuring more than adequate sterilisation, and preserving the original structure. Such porous materials when used in implants would permit circulation of body fluids, allowing firm attachment of living tissue, to enable regeneration as rapidly as possible

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Non-electrolyte Probes of Membrane Structure in ADH-treated Toad Urinary Bladder

ALTHOUGH it is well known that antidiuretic hormone (ADH) increases the permeability of amphibian urinary bladders to water and small polar non-electrolytes such as urea and acetamide[1,2], there is much uncertainty about the mechanisms involved. To gain further insight into the interaction of ADH with this membrane, we have studied the permeation of lipophilic molecules in the presence and absence of the hormone. It has long been recognised that permeability measurements may offer unique clues about the nature of biological membranes[3,4].

Our approach was similar to that used previously to study non-electrolyte permeation across the gall bladder[5]. Urinary bladders from the toad (*Bufo marinus*) were mounted as flat sheets between two lucite chambers. The area of the window between the chambers was 1.3 cm^2, and each chamber contained 16 ml of saline. The composition of the saline was 104.5 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$ and 1 mM $MgSO_4$ buffered at *p*H 7.4 with 2.125 mM Na_2HPO_4 and 0.375 mM NaH_2PO_4. The saline in each chamber was stirred vigorously with bar magnets, and was gassed with 100% oxygen. The electrical resistance of the urinary bladders ranged from 1,400 to 6,500 ohms cm^2 with a mean value of 2,600±70 (150) ohms cm^2. All experiments were carried out at room temperature (22°–24° C).

The permeability of lipophilic non-electrolytes, that is those with an oil/water partition coefficient greater than 1×10^{-3}, was determined by radioactive tracer techniques. A tracer quantity of the radioactive solute was added to the mucosal solution, and the rate of appearance of the isotope in the serosal solution was determined by withdrawing aliquots of the serosal solution at regular intervals. All radioactive samples were assayed by conventional liquid scintillation counting techniques. Permeability coefficients (*Ps*) were expressed in cm s^{-1}, and were corrected for unstirred layer effects whenever necessary[5].

Radioactive ^{14}C-isotopes were purchased from International Chemical and Nuclear Co., Irvine, California (1,4-butanediol, 1,2-propanediol, antipyrine, caffeine, 1,7-heptanediol, and 1,6-hexanediol), Amersham/Searle Corp., Arlington Heights, Illinois (nicotinamide) and American Radiochemical Corp., Sanford, Florida (n-butyramide and iso-butyramide).

For these non-electrolytes, the unidirectional fluxes reached a steady state within 1 h of addition of the isotope, and remained constant for at least 6 h. ADH ('Pitressin') was added to the serosal solution 2 h after the addition of the isotope to give a final concentration of 130 mU ml^{-1}. *Ps* of the untreated bladders were calculated from the fluxes during the hour before the addition of ADH, and the *Ps* in the presence of ADH were calculated from the fluxes measured in the hour following addition of the hormone.

In the absence of ADH the permeabilities varied from 12 ± 1 (8)$\times10^{-7}$ cm s^{-1} for nicotinamide to 290 ± 11 (6)$\times10^{-7}$ cm s^{-1} for caffeine. As anticipated, there was a linear relationship between the permeability coefficients of the lipophilic solutes and their olive oil/water partition coefficients: the slope of the line was 1, and the regression coefficient was 0.86. This indicates that the membrane, or membranes, controlling permeation across this epithelium are as hydrophobic as olive oil, and that the selectivity for both permeability and bulk phase partition coefficients is governed by the same basic intramolecular forces[4].

Addition of ADH caused a statistically significant increase in the permeation of all solutes across the bladder. The *Ps* for nicotinamide and caffeine increased to 23 ± 2 (6)$\times10^{-7}$ and 377 ± 0 (2)$\times10^{-7}$ cm s^{-1} respectively. The *Ps* for all nine non-electrolytes in the presence of ADH are plotted against those in the absence of ADH in Fig. 1. This shows that there is a linear relation between the permeabilities in the absence and presence of hormone; the slope of the line was 1.3, and the regression coefficient was 0.99. This leads to the conclusion that the hormone produces a generalised, but unselective, increase in the permeability of all the lipophilic compounds, that is the permeability of all the compounds increased by roughly the same factor, and there is no apparent change in the selectivity. (Similar changes in permeability were produced by the addition of ADH to the mucosal solution, but only in the presence of 0.5–1 mM $LaCl_3$. This suggests that the surface charge on the membrane surface is involved in the action of the hormone.)

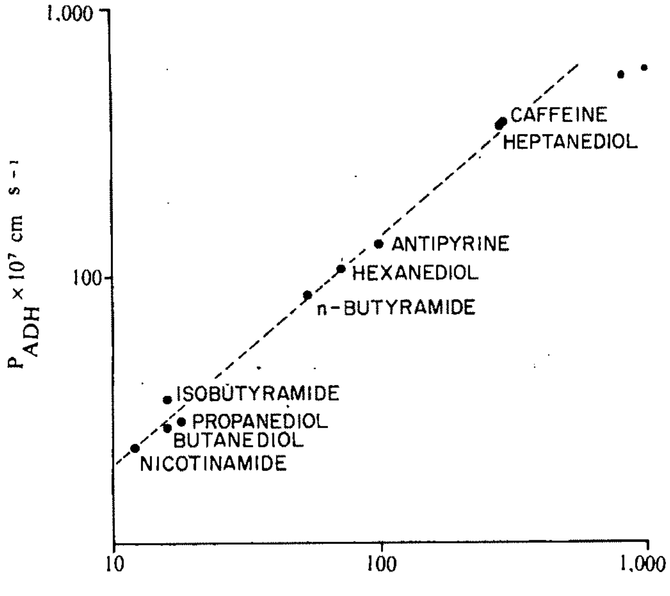

Fig. 1 The effect of ADH on the permeation of lipophilic solutes across the toad urinary bladder. Non-electrolyte permeability coefficients (cm s^{-1}) obtained in the presence of ADH (on the ordinate) are plotted against the coefficients obtained in the absence of the hormone (on the abscissa).

The effect of ADH on the permeability of the toad bladder to lipophilic solutes is much smaller than that obtained with hydrophilic solutes. For example, the permeabilities of urea, acetamide and water increased from 14 ± 1 (16)$\times10^{-7}$, 16 ± 1 (44)$\times10^{-7}$, and $1,300\pm80$ (56)$\times10^{-7}$ cm s^{-1} to 49 ± 8 (2)$\times10^{-7}$, 72 ± 16 (14)$\times10^{-7}$ and greater than $10,000\times10^{-7}$ (8) cm s^{-1} respectively.

Many biological membranes discriminate between branched and straight chain isomers[4]. Branched chain solutes are usually much less permeable than the straight chain isomers, and this has been attributed to the highly ordered configuration of the lipids in the cell membrane. In the toad bladder we find that in the absence of ADH the permeability of iso-butyramide is 16 ± 1 (20)$\times10^{-7}$ cm s^{-1}, whereas the permeability of n-butyramide is 54 ± 3 (20)$\times10^{-7}$ cm s^{-1}. This difference in permeability is strikingly similar to that for the permeation of the amides across red cell membranes[6] and gall bladder epithelium (unpublished observations). Thus it may be concluded that the lipids exist in a highly ordered configuration in the epithelial membranes of the toad urinary bladder.

The permeability of both isomers increased when ADH was added, but the increase obtained with iso-butyramide was much more dramatic than with the straight chain isomer. The permeabilities of iso-butyramide and n-butyramide increased to 35 ± 3 (8)$\times10^{-7}$ and to 68 ± 7 (8)$\times10^{-7}$ cm s^{-1} respectively. In paired experiments on tissues from the same animal, the ratio of the iso-butyramide to n-butyramide permeabilities increased from 0.30 to 0.51 when the membrane was treated with ADH. These results strongly suggest that the branched molecule encounters less steric hindrance in permeating across ADH treated membranes than in control membranes. This leads to the conclusion that ADH increases the permeability of the toad urinary bladder epithelium by increasing the fluidity of the cell membranes.

In biological and artificial membranes it has been reported previously that increases in membrane fluidity are associated with positive increments in non-electrolyte permeation[7–10]. Furthermore, recent studies of liposomes showed that when the hydrocarbon tails of the membrane lipids passed from a fluid into a crystalline state, there was a decrease in the partition of solutes into the membrane[11–14]. This suggests that ADH effects in the toad bladder epithelium result from an increase in the partition of solutes into the plasma membranes, but changes in solute mobility cannot be excluded.

It is now recognised that the partition of solutes into biological and artificial membranes probably varies with position in the membrane; lipophilic solutes are expected to be partitioned mainly into the hydrocarbon core of the membrane, whereas hydrophilic solutes are probably located nearer the polar head groups of the lipids[13]. Consequently, changes in fluidity of a membrane could produce quantitatively different effects on lipophilic and hydrophilic solutes. If, for example, ADH caused a relatively larger increase in the fluidity at the periphery than in the core of the bladder membranes, there should be a greater increase in the permeation of hydrophilic solutes than lipophilic solutes. Such an effect may, at least partially, account for the quantitative difference between the effect of the hormone on small polar solutes and lipophilic solutes. This hypothesis could be tested by measuring the effect of the hormone on the partition of solutes into isolated plasma membranes of the toad bladder.

It is still not certain whether or not ADH acts on the apical cell membrane of the toad bladder epithelium directly, or through some intracellular mediator. Evidence for a direct effect on the apical membrane, at least for non-electrolyte permeability, is that (i) vasopressin increases the osmotic water permeability of artificial bilayer membranes, whereas cyclic AMP does not[15–17], and (ii) ADH in the mucosal solution increases the permeability of the toad bladder when the surface charge on the outer surface of the apical membrane is screened with polyvalent cations.

In conclusion, we have shown the ADH increases the permeability of the toad urinary bladder to lipophilic solutes, in addition to the effect on small polar solutes and water. This increase in permeability is associated with an increase in the fluidity of the membrane, or membranes, controlling permeation across the epithelium. Evidence is cited to suggest that the change in configuration of membrane lipids acts to increase the partition of solutes into the membrane, and that quantitatively different effects are expected for lipophilic and hydrophilic solutes. These experiments emphasize that a direct interaction of the ADH with the membranes of the toad urinary bladder may account for at least some effects of this hormone. Further clues will undoubtedly come from experiments designed to evaluate the effect of ADH on permeability of the apical membrane of the epithelium.

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Melatonin stimulates Growth of Brown Adipose Tissue

IN small mammals brown adipose tissue (BAT) is an important site of non-shivering heat production[1,2]. Its mass increases during cold adaptation together with the ability to produce heat (ref. 3 and G. H., unpublished), thus improving cold tolerance[4]. In the golden hamster, Hoffman *et al.*[5] also found an increase of BAT weight following exposure to short photoperiods. The pineal hormone melatonin has been shown to affect photoperiodic responses (ref. 6, and K. H. and G. H., unpublished), and therefore an action of melatonin on development of BAT seemed likely. In this study, the effect of short photoperiods and of melatonin on the amount of BAT was investigated in the Djungarian hamster *Phodopus sungorus*. This species shows a marked annual cycle of testis size and activity, body weight, and coat colour when kept under natural light conditions[7], and all of these functions can be influenced by manipulation of the photoperiod (ref. 6, and K. H. and G. H., unpublished). The species does not hibernate, but may show daily torpor in winter, while the gonads are regressed even at room temperature[7].

Fifty adult male hamsters (average body weight 42 g) that had been kept under natural light conditions and temperatures of 20±3° C were exposed to either long (16 h light d^{-1}) or short (8 h light d^{-1}) artificial photoperiods at 20±1° C starting in July. The animals were housed singly. Nine hamsters in either condition were implanted with tablets of one part melatonin in ten parts beeswax (average weight of implants 34 mg) subcutaneously six times at weekly intervals. Each tablet thus contained about 3.1 mg of melatonin but control experiments showed that only a small fraction of this was absorbed[6]. Control animals in each light condition were either left untreated (seven hamsters each), or received implants of beeswax only (nine hamsters each). After 64 d under artificial light conditions, the hamsters were killed, weighed, and stored in a deep freeze until dissection. Drying was prevented by wrapping each animal in a plastic bag. Body weight was again checked before removal of BAT and no decrease resulting from storage was found. BAT was carefully dissected from the interscapular, subscapular, dorsal, cervical, suprasternal, subvertebral and other intrathoracal sites and was weighed immediately after dissection. No BAT was taken from the perirenal region.

The weight of BAT thus determined is given in Fig. 1 for all hamsters. To eliminate the effect of differences in body weight, weight of BAT was calculated as a percentage of body weight. Control hamsters kept the 8 h photoperiod contained nearly 50% more BAT than those kept in the 16 h photoperiod ($P<0.001$; t test). Hamsters that had received melatonin had the highest amount of BAT; on the average it was more than 5% of body weight. In both photoperiods, BAT mass of hamsters treated with melatonin differed significantly from that of the respective controls ($P<0.001$). No difference in BAT content was found in the two groups receiving melatonin but kept in different photoperiods.

The finding that the controls contained significantly higher amounts of BAT after exposure to short photoperiods confirms results obtained in the golden hamster[5], and shows that seasonal increase in BAT mass in winter is not simply a consequence of cold exposure, but can also result from the short photoperiod at that season.

The drastic increase of BAT mass in hamsters treated with melatonin indicates that the pineal may be involved in its regulation. The pineal has been shown to participate in the regulation of seasonal cycles by light[8,9]. In mammals, melatonin is exclusively produced in the pineal, and the activity of hydroxyindole-O-methyltransferase, the enzyme responsible for the last step in melatonin formation, has been reported to be light dependent, showing high activity in darkness and low activity in light[8]. Our data demonstrate a drastic effect of melatonin on growth of BAT, and it seems reasonable to assume that the rise in BAT content in hamsters kept in short photoperiods might be mediated by increased production of melatonin in the pineal in these conditions. As no difference in BAT was found between the two photoperiods in the hamsters treated with melatonin, and as the amount was nearly twice that found in the controls in short photoperiods, one might speculate that the amount of melatonin effective in our experiments already instigated the maximal possible growth of BAT, and that therefore no additive effects of photoperiod could be observed. This assumption is supported by the fact that the amount of BAT found in our experiments after melatonin treatment is higher than nearly all previously reported values[1].

The pathway by which melatonin acts on the growth of BAT is unknown. Melatonin has been claimed to be the antigonadotrophic hormone of the pineal[8] but contradictory results have been obtained after its administration[9,10]. In *Phodopus*, extraneously administered melatonin delayed the accelerating effect of long photoperiods on testis development, increase of body weight, and change into summer coat in midwinter, but did not completely suppress such development[6]. In summer, melatonin suppressed or greatly diminished the effect of short photoperiods which in untreated controls caused testicular involution, decrease of body weight, and change into winter coat (K. H. and G. H., unpublished). In the experiments reported here, however, melatonin caused enlargement of BAT independent of photoperiod. Thus, its action does not parallel that on the

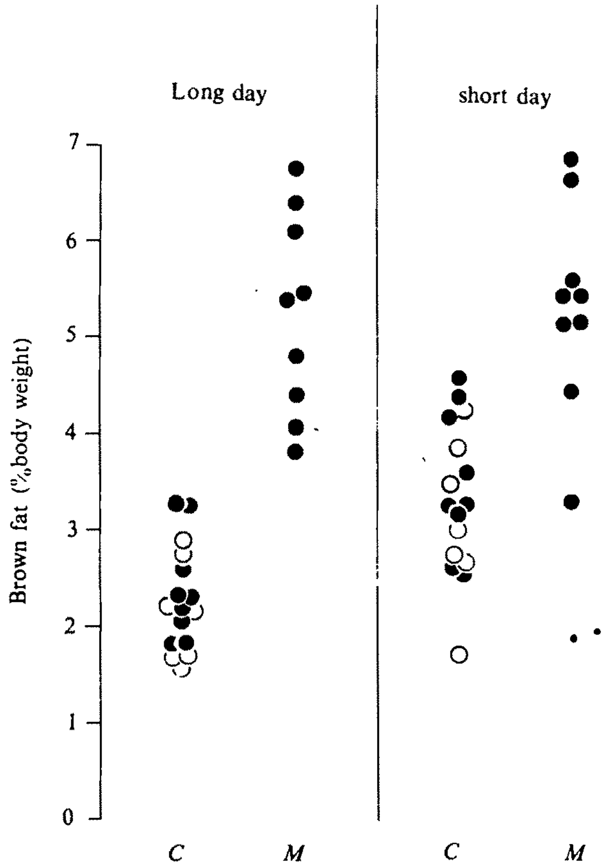

Fig. 1 Weight of brown adipose tissue (BAT) as percentage of body weight in the Djungarian hamster *Phodopus sungorus*. Each point represents BAT weight of a single hamster. *M*, Hamsters treated with melatonin; *C*, controls without any treatment (○) and implanted with plain beeswax (●). Mean ±s.e.: long day, *C*, 2.30±0.13; *M*, 5.24±0.35; short day, *C*, 3.34±0.20; *M*, 5.33±0.36. Long day, 16 h light; short day, 8 h light.

other functions showing seasonal variations, and must be brought about by different pathways.

The physiological mechanism controlling development of BAT during cold adaptation is not resolved. There are indications that thyroxine and noradrenaline are involved in stimulation of BAT growth because, in rats, an increase in BAT mass was observed after repeated injections of thyroxine and/or noradrenaline[4]. Blocking the thyroid activity in mice by feeding them with propriothiouracil caused a reduction in the amount of BAT (G. H., unpublished). In young rats, growth of BAT was stimulated by injections of cortisone[11]. Little is known about the effects of melatonin on the adrenal cortex, but it has been reported to inhibit thyroid function, though the results are far from uniform[8,12]. As melatonin stimulated BAT growth in our experiments it does not seem to act by way of the thyroid. A direct effect on BAT growth or a completely different pathway seems more likely.

The action of melatonin on growth of BAT described here is, to our knowledge, the first evidence that the pineal may be involved in thermoregulatory adjustments in mammals.

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Transmission of Mammalian Piroplasm by an Argasid Tick

IT is the accepted view that parasites belonging to the suborder Piroplasmidae Wenyon, 1926, are exclusively transmitted by "hard" ticks belonging to the family Ixodidae Murray[1,2]. Most piroplasms of domestic animals have more than one Ixodid vector. In the laboratory, small mammal piroplasms belonging to the genera *Babesia* Starcovici, 1893, and *Nuttallia* Franca, 1910, are used as models for the diseases caused by piroplasms of domestic animals. This research, however, suffers from the drawback that the parasites cannot be biologically transmitted, except in two cases. Thus, *Babesia rodhaini* Van den Berghe, Vincke, Chardome and Van den Bulcke, 1950, a rodent piroplasm very commonly used in laboratory investigations, has no known vector; nor has *Babesia hylomysci* Bafort, Timperman and Molineux, 1970. The two small mammal piroplasms of which the vectors are known are *Nuttallia microti* Coles, 1914, and *Nuttallia danii* Tsur, Hadani and Pipano, 1960 (and the probably identical *N. tadzhikistanica* (Krylov and Zanina, 1963), Krylov, 1964). The vectors of these piroplasms are *Ixodes trianguliceps*[3] Birula, 1895, for the former, and three species of *Hyalomma* and two species of *Rhipicephalus*[4] for the latter. All of these vectors belong to the Ixodidae.

Nuttallia meri Gunders, 1971, was isolated from the fat sand rat *Psammomys obesus* Cretzchmar, 1828, from the lower Jordan Valley[5] and, though having a narrow range of infectivity for laboratory animals, is considered a potential model for the study of piroplasmosis of economic importance. The search for possible tick vectors in nature led to the discovery, within *Psammomys* burrows, of the "soft" tick *Ornithodoros erraticus* (Lucas, 1849) (small race) which had not previously been reported from Israel[6]. No other ticks were found in this biotope.

We have reported elsewhere[7] failure to transmit *N. meri* through clean laboratory-raised *Hyalomma excavatum* Koch, 1844, *Rhipicephalus sanguineus* (Latreille, 1806) and *Ornithodoros tholozani* (Labulbène and Megnin, 1882), but successful infection of clean laboratory-bred, splenectomised, *Psammomys* (HUPO strain) followed intraperitoneal injection of triturated field-collected *O. erraticus*. Further, one laboratory-bred *Psammomys* became infected with *N. meri* after eleven adult ticks were fed on it. The ticks had been collected in the field 2 d earlier. Although there had been a lapse of only 2 d between field collection and biting by the ticks, it was clear that this was true transmission, as the ticks had to be starved to be ready to feed.

The purpose of this communication is to report three further instances of transmission of *N. meri* to *Psammomys* by the bite of *O. erraticus* up to 81 d after field collection of the ticks. All three sand rats were of the Hebrew University strain of *Psammomys obesus*, were laboratory bred and were splenectomised before having ticks fed on them. Four nymphs and three imago *O. erraticus* that had been collected in the field 39 d earlier were fed on the first *Psammomys*. Parasitaemia became apparent on day 17. Eight adult *O. erraticus* and one nymph were fed on the second sand rat 81 d after their collection. The sand rat became manifestly infected on day 17. A pool of starved ticks were fed on the third *Psammomys*. The ticks included some that had infected the first *Psammomys* on day 39 after collection and others that had been found to be uninfected. Again, transmission of *N. meri* occurred and was observed on day 12, indicating that a batch of ticks may infect more than once and that the parasite persists in the ticks, even after a blood meal, for at least 81 d.

The four instances of transmission, by bite, of *N. meri*, through field-collected *O. erraticus* clearly establish this tick as a natural vector of this parasite. This is the first proven example of transmission of a mammalian piroplasm by an Argasid ("soft") tick. The reports by Rastegaïeff[8] and Bitukov[9] suggesting that *Theileria* (=*Gonderia*) *ovis* (Rodhain, 1916) Lestoquard, 1929, was transmitted through *O. lahorensis* Neumann, 1908, are considered doubtful by Neitz (ref. 1 and personal communication) because of the excessively short incubation periods following tick bite (3 and 8 d, respectively) and because the sheep and goats had not been shown to be free of naturally acquired infection before their use as experimental animals.

The demonstration that an Argasid tick, *Ornithodoros erraticus*, could transmit, by bite, a mammalian piroplasm (*N. meri*) suggests that, for those small mammal piroplasms for which no natural vector is as yet known, a search among the soft ticks may be rewarding.

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Carnosine in Nucleated Erythrocytes

IN the course of studies of the metabolism of intracerebrally administered L-U-^{14}C-histidine in brains of mice and frogs (*Rana pipiens*), analyses were also performed on blood samples from the injected animals. The mice received 1.5×10^6 c.p.m. of the labelled histidine, and 1 h later samples of blood were taken by means of orbital puncture. Frogs were injected with 2.9×10^6 c.p.m. and blood was obtained after 1 h by cardiac puncture. The samples were deproteinised in 5 volumes of 0.4 N perchloric acid and processed in the manner described previously for brain and tumour tissues[1]. Aliquots of neutralised extracts were electrophoresed on Whatman No. 3 paper at *p*H 10.0 together with known standards of carnosine, homocarnosine, histidine, imidazoleacetic acid, N-acetylhistidine, and histamine; and stained with Fast Red B in the manner described previously[1].

Scanning the electropherograms showed only a few very minor radioactive peaks, which were not investigated further, but a marked enhancement of the size of the carnosine marker spot was noted in the extracts of frog blood. Electrophoresis without added markers showed a strongly diazo-positive material to be present in the carnosine position in extracts of frog blood, and subsequent experiments showed it to be largely located in the erythrocytes. Electropherograms of mouse blood were negative.

When neutralised perchloric acid extracts of frog erythrocytes were subjected to two-dimensional thin-layer chromatography (TLC) (ref. 2), the major diazo-positive substance migrated to a position corresponding to that of carnosine (Fig. 1). In addition, there was a small amount of material corresponding in position to N-acetylhistidine on the chromatograms. We have shown previously that, in the frog, intracerebrally injected histidine is converted extensively into N-acetylhistidine[1]. When the frog erythrocyte extracts were chromatographed with histidine, carnosine, homocarnosine and N-acetylhistidine (Fig. 2), it became apparent that the unknown material was probably carnosine. Material containing the compound was then isolated from one-dimensional TLC plates[2] and a portion was hydrolysed at 110° C in 6 N HCl for 20 h as shown by two-dimensional TLC (ref. 2) of equal aliquots of hydrolysed and unhydrolysed samples. Disappearance of the original material and liberation of β-alanine and histidine were noted on hydrolysis (ref. 2).

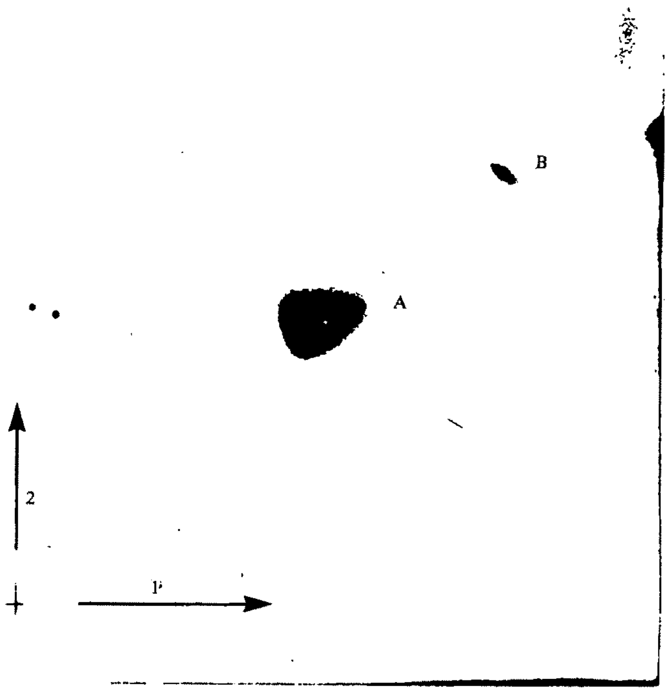

Fig. 1 Two-dimensional TLC on silica gel[2] of extract corresponding to 20 µl of packed frog erythrocytes. The chromatogram was run in the first dimension (1) in ethanol : ammonia (28%) : water (70 : 5 : 25) and in the second dimension (2) in chloroform : methanol : ammonia (28%) : water (30 : 60 : 5 : 15). Spot A occurs in a position characteristic for carnosine, and spot B for N-acetylhistidine[2].

Analysis of the above samples by column chromatography on a Beckman amino acid analyser showed there to be 38 nmol of histidine and no β-alanine in unhydrolysed extract corresponding to 1 ml of packed erythrocytes, while after hydrolysis 116 nmol of histidine and 92 nmol of β-alanine were found. Thus, histidine and β-alanine were liberated in approximately isomolar amounts on hydrolysis, confirming the identity of the diazo-positive material with carnosine.

Employing the above techniques, carnosine has been detected in erythrocytes of White Leghorn chickens, but in smaller quantities than in the frog. To date we have not

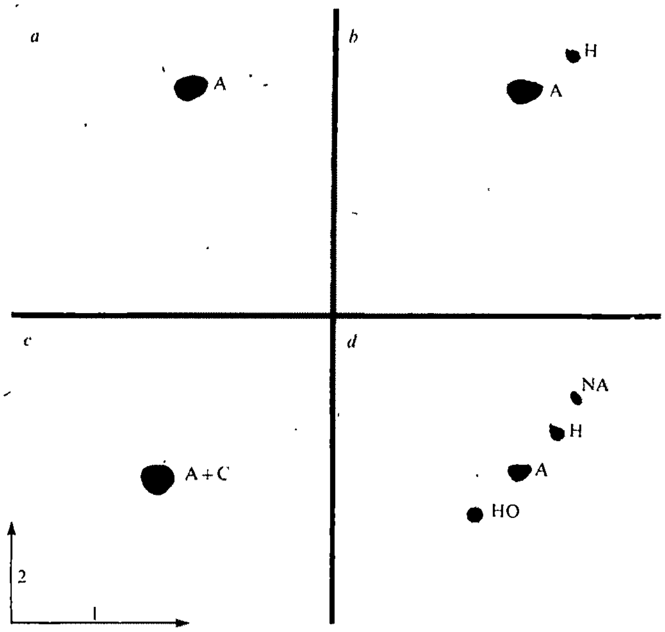

Fig. 2 Two-dimensional TLC of equal amounts of isolated spot A with and without markers run in the same manner as in Fig. 1. Chromatograph *a*, spot A alone; *b*, histidine added; *c*, carnosine (c) added; *c*, together with homocarnosine (HO), histidine (H), and N-acetylhistidine (NA). Solvent system same as for chromatograph of Fig. 1.

been able to detect carnosine in concentrated extracts of mouse erythrocytes.

To our knowledge, carnosine has not been detected previously in erythrocytes of any species. Our results here show the presence of this dipeptide in relatively large amounts in frog erythrocytes and in smaller amounts in chicken erythrocytes, although it was undetectable in mouse red blood cells. Meaningful suggestions about the source or function of carnosine in nucleated erythrocytes cannot be made until much further work is accomplished. Although the presence of carnosine in muscle has been known for over 70 years[3], its function is still largely shrouded in mystery.

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Association of Methionine Requirement with Methyl Mercury Resistant Mutants of Yeast

It has been known for several years that strains resistant to mercury can be obtained in several bacterial species[1–4]. Soon after the correlation between resistance to antibiotics and to mercury was recognised[2,5], it was established that genetic elements conferring resistance to antibiotics, mercury and other heavy metals in *Escherichia coli* and *Salmonella typhimurium*[6] and *Staphylococcus aureus*[7,8] reside on extrachromosomal resistance transfer factors or plasmids. Among fungi, mercury resistant strains of *Botrytis cinerea*[9], *Penicillium notatum, Sclerotinia fructicola, Stemphylium sarcinaeforme*[10], and *Saccharomyces cerevisiae*[11,12] have been reported. In most cases[1,3,9–12], this was accomplished by 'training' the normal strains for growth on media supplemented with successively increasing concentrations of mercury compounds, and in some cases[10] the resistance was lost when subcultured on mercury-free media. It is noteworthy that in none of the mercury-adapted strains of fungi has the genetic basis of resistance been determined. In this report we describe a method of isolation and characterisation of methyl mercury resistant mutants of *S. cerevisiae*. This study was undertaken with the view that the examination of physiological changes associated with genetically defined resistant mutants will be useful in studying the mechanisms of cellular detoxification of organic mercurials.

We used the following simple technique as the most efficient means of isolating spontaneous and induced methyl mercury resistant mutants. Fresh stationary phase cultures of haploid strains in YPD medium (1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose) were washed and treated with 3% ethyl methanesulphonate for 1 h at 30° C. From 5×10^6 to 1×10^7 treated cells were spread on each dish of minimal agar medium (0.67% Bacto-yeast nitrogen base without amino acids, 2% dextrose and 1.5% agar) supplemented only for the nutritional requirement of the original strains. Sterile paper strips or disks were placed in the middle of the plates on which 30 to 60 nmol (100 to 200 μl of a 3×10^{-4} M solution) of methyl mercury chloride (K and K Laboratories, Plainview, New York) was dispensed. The cells were thus exposed to a continuous concentration gradient of methyl mercury. The plates were incubated at 30° C for 7–10 d. Cells could grow into a confluent lawn at the edge of the plate where the methyl mercury concentration was low and not inhibitory, but they could not grow near the impregnated paper. Occasionally a few colonies appeared in a region where the methyl mercury concentration was inhibitory to most cells. These colonies were isolated as presumptive resistant mutants and tested further. We have also isolated spontaneous and ultraviolet light-induced mutants by this method.

When colonies selected for methyl mercury resistance were analysed, most of them would not grow on minimal medium with or without methyl mercury and with the supplements necessary to satisfy the nutritional requirement of the parent strain. Further tests revealed that these mutants had become auxotrophic for methionine. It should be noted that the selection medium did not contain methionine. Furthermore, the addition of methionine to the selection medium resulted in a marked decrease of methyl mercury resistant mutants.

Eight mutants were crossed to normal strains, the diploid cells were sporulated, and genetic analysis was performed on the resulting meiotic segregants by standard procedures. In all eight cases, the methionine requirement segregated in the manner expected of a single chromosomal gene.

We then performed complementation tests with all pairwise combinations of the eight mutations. From this and from analysis of crosses among the mutants, it was established that all eight were allelic mutations. To determine whether the genetic lesions responsible for the requirement of methionine in the methyl mercury resistant strains arose at one of the previously identified genes concerned with methionine biosynthesis or whether they occurred at a previously undetected locus, complementation tests were carried out between this *met* gene and the standard methionine tester strains obtained from the Yeast Genetics Stock Center, University of California, Berkeley. These *met* mutants complemented mutants in all the previously identified *met* genes represented in the stock collection, *mes1, met1, met2, met4, met5, met6, met7, met8, met10, met13,* and *met14,* as well as *met3* which was isolated in our laboratory. (As far as we know, the *met9, met11* and *met12* mutants do not exist or represent alleles of some of the above loci (see refs 13 and 14).) To rule out the possibility of interallelic complementation between our *met* mutations and any of the other known *met* genes, a methyl mercury resistant strain was crossed to each of the methionine testers individually. Tetrad analyses of spore clones from these crosses showed that the *met* mutations in our strains segregate independently of all available previously known genes involved in methionine biosynthesis. We have designated this new locus *met15*.

We could not demonstrate resistance by comparing growth of the normal strain and *met15* strains on media containing various concentrations of methyl mercury or containing gradients of methyl mercury. Perhaps the differences are too slight to be revealed by these methods. The resistance of *met15* strains to methyl mercury, however, can be unequivocally shown by a reconstruction experiment (Fig. 1).

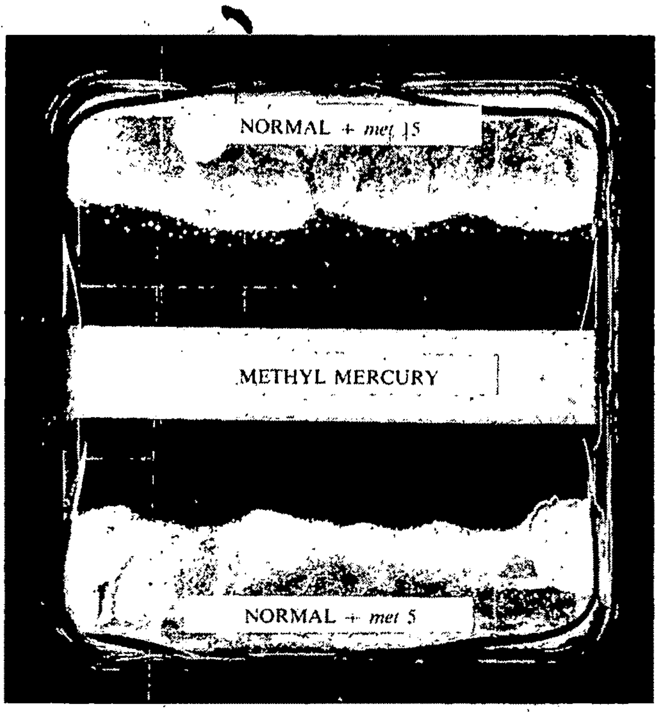

Fig. 1 Methyl mercury resistance of *met15* strains. The upper half of the plate was spread with approximately 2×10^6 cells from a mixed suspension of normal +*met15* strains and the lower half with 2×10^6 cells from a suspension of normal +*met5* strains. The ratio of normal to *met* cells in both cases was about 1,000 : 1. The paper strip in the middle was impregnated with 100 µl of 3×10^{-4} M solution of methyl mercury chloride. The photograph was taken after 7 d of incubation at 30° C. The methyl mercury resistant colonies of the *met15* strain (upper) can be seen growing before the front of the lawn of the normal strain.

The normal strain AS2–2C (α *leu2–1*) and the mutant S4 (α *leu2–1 met15–4*) obtained from AS2–2C were grown separately, washed and mixed. In addition a mutant S46 (α *leu2–1 met5*), directly isolated by replica plating techniques from AS2–2C, was similarly grown and mixed with the normal strain. The two kinds of mixed populations, as described in the figure legend, were plated on minimal agar medium supplemented only with leucine and exposed to methyl mercury. Only in the section of the plate seeded with normal+*met15* cells did any resistant colonies appear. These colonies could be isolated and analysed further. In one such reconstruction experiment 154 of the 164 isolated colonies carried the *met15* gene, and more importantly, 149 of the 154 *met15* colonies carried the input allele, *met15–4*. The remaining five were apparently new mutations at the *met15* locus. The frequency of recovery of the input *met15* strain was markedly reduced if the experiment was done on a medium supplemented with leucine and methionine. In the experiment described above, only fourteen *met15–4* colonies could be recovered from the medium containing leucine and methionine. Thus methionine inhibited the expression of resistance, a phenomenon corroborated by the decrease in mutation frequency on methionine-supplemented medium.

An interesting property of the resistant mutants was uncovered when we examined the interrelationship between methyl mercury resistance and methionine auxotrophy by growing the mutants on double gradient plates of methyl mercury and methionine (Fig. 2). The gradients were generated by adding the methyl mercury and methionine on paper strips which were placed at right angles to each other along the edges of the plates. Figure 2 shows growth patterns of S4 (α *leu2–1 met15–4*) and S46 (α *leu2–1 met5*). As noted earlier, the two strains are isogenic except for their *met* genes. The methionine auxotroph (*met15*), which is resistant to methyl mercury, has a growth pattern very different from the other methionine auxotroph (*met5*). We have so far compared the growth patterns of five different *met15* mutants with those of controls consisting of strains carrying mutations at *met5, met8* or *met10* locus, but otherwise isogenic to the *met15* strains. In every case, the *met15* strain showed the growth pattern depicted in Fig. 2*a*, whereas the control exhibited the pattern represented in Fig. 2*b*. We interpret the peculiar growth response of *met15* strains on the double gradient plates to indicate that at a critical concentration of methyl mercury the methionine requirement of the *met15* mutants is partially or completely alleviated. It also appears from the growth pattern on the *met5* plate that methionine at higher concentrations may be preventing methyl mercury toxicity.

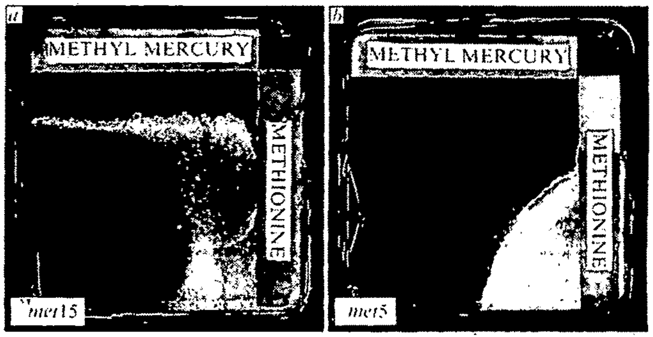

Fig. 2 Growth patterns of a *met15* (*a*) and a *met5* (*b*) strain on double gradient of methyl mercury and methionine. Approximately 1.3×10^6 cells per plate were spread uniformly over the whole area of the plates which contained minimal agar medium supplemented with leucine; 100 µl of 3×10^{-4} M solution of methyl mercury chloride and 200 µl of 1.6×10^{-2} M solution of methionine were used on the respective strips. The photographs were taken after 8 d of incubation at 30° C. The protruding area of growth of the *met15* strains indicates that the methionine requirement was completely or partially alleviated at certain concentrations of methyl mercury.

The data so far are consistent with the following interpretation of the interrelationship of methionine requirement and methyl mercury resistance. We hypothesise that a compound involved directly or indirectly in the biosynthesis of methionine can cause detoxification either by the formation of a complex with methyl mercury or by conversion of it to a volatile product. This compound accumulates in *met15* mutants and inhibits methionine biosynthesis. The diminution of the methionine requirement of the resistant mutants in the presence of methyl mercury may then be explained by the destructive action of methyl mercury on this compound. Methionine may inhibit the production of this compound by repression or feedback inhibition which would explain the poor expression of the resistance in the presence of methionine. These yeast mutants do not seem to detoxify methyl mercury by the same mechanisms found in other mercury resistant organisms[15–18]. We are continuing the investigation in the hope of explaining the results at molecular level as well as to test our working hypothesis.

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Free and Occluded Virus, similar to *Baculovirus*, in Hepatopancreas of Pink Shrimp

VIRUSES or virus-like particles have been reported infrequently from cells of estuarine and marine organisms[1–4]. Herpes-type viruses were described from an estuarine fungus[3] and oysters[4]. Helical or rod-shaped viruses have not been reported from most aquatic invertebrates, although rod-shaped virus-like particles were reported from a microannelid[5] and aquatic beetle[6].

Recently, I observed rod-shaped virus particles and related inclusion bodies in cells of pink shrimp (*Penaeus duorarum*) that were experimentally exposed to the polychlorinated biphenyl (PCB), Aroclor 1254. This report describes these particles and demonstrates their similarity to certain non-inclusion viruses and to nuclear polyhedrosis viruses of the *Baculovirus* group previously described only from insects and mites[6–10].

Adult pink shrimp, from the Gulf of Mexico near Cedar Key, Florida, were kept in flowing seawater and exposed to 3–5 μg l^{-1} PCB for 30–52 d as described elsewhere[11]. Control shrimp from the same stock were maintained in PCB-free flowing seawater. Non-experimental pink shrimp which were collected periodically from near Cedar Key and Pensacola, Florida, and prepared immediately for cytology are considered here as feral or wild shrimp. Hepatopancreatic tissues from forty exposed shrimp, forty control shrimp and fifty feral shrimp were fixed for histology in Davidson's fixative[12], sectioned and stained with Harris haematoxylin and eosin, mercury bromophenol blue or the PAS method.

Hepatopancreatic tissues from five exposed, five control and ten feral shrimp were fixed for electron microscopy

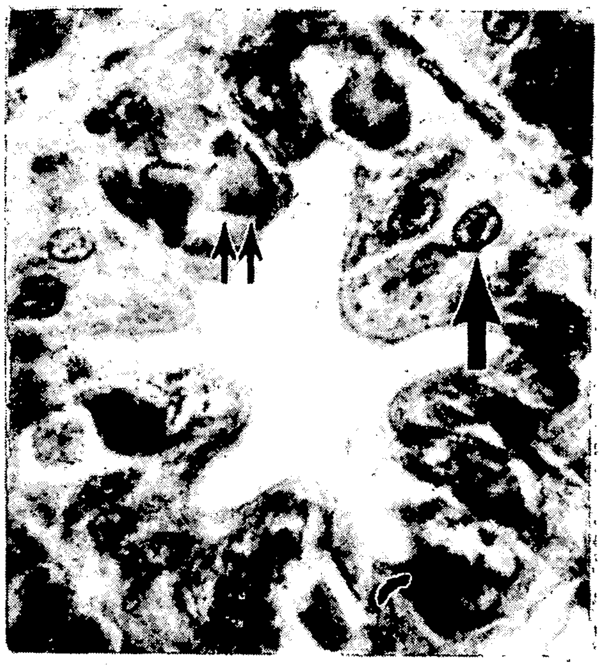

Fig. 1 Light micrograph of cross section of hepatopancreatic tubule from pink shrimp exposed to PCB; note normal nucleus of epithelial cell (one arrow), and crystalloid, tetrahedral inclusion bodies in hypertrophied nucleus of affected cells (two small arrows).

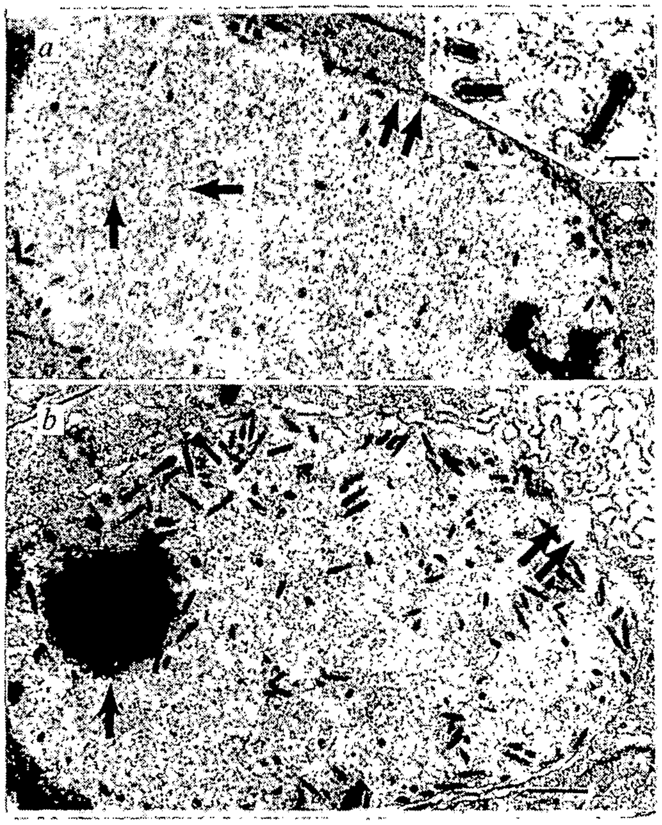

Fig. 2 *a*, Hypertrophied nucleus in epithelial, absorptive cell from hepatopancreas of shrimp exposed to PCB; non-occluded rod-shaped virions in longitudinal and cross section; single arrows point to intranuclear membranes associated with early virion formation, double arrows indicate abnormal multilaminate nuclear envelope. Inset: high magnification of rod-shaped virion in nucleus (bar=0.1 μm); note single envelope surrounding nucleocapsid. *b*, Moderately heavily affected nucleus; virions distributed mostly around inner periphery of nucleus; arrow points to degenerate nucleolus; double arrows point to envelope membranes entering the cytoplasm. Note many free ribosomes in cytoplasm (bar=1 μm).

(2.5% glutaraldehyde and 1% osmium) and embedded in Epon 812. Sections 50–100 nm thick were cut, collected on unsupported 300 mesh copper grids and stained with uranyl acetate and Reynold's lead citrate according to the short method of Hayat[13]. Approximately 3,000 hepatopancreatic cell profiles from feral and control shrimp and 1,500 hepatopancreatic cell profiles from exposed shrimp were examined with the electron microscope.

Light microscopy of fresh squashes and fixed, stained hepatopancreatic tissues of control shrimp revealed normal histological and cytological structure. Thirty per cent of shrimp exposed to PCB, and approximately 20% of feral shrimp from near Cedar Key, however, had crystalloid inclusion bodies in distorted, hypertrophied nuclei of hepatopancreatic epithelial cells (Fig. 1). These nuclear inclusion bodies were usually tetrahedral (pyramidal) in form in fresh squashes and triangular in sections. They ranged in size from 0.5 μm to 12 μm from base to point, and stained light blue with mercury bromophenol blue, and were PAS-negative.

Electron microscopy demonstrated normal ultrastructure of hepatopancreatic tubule cells and nuclei in feral and control shrimp. However, approximately 8% of the nuclear profiles of hepatopancreatic tubule cells from three PCB-exposed shrimp were hypertrophied, and contained rod-shaped, free (non-occluded) and occluded virions (Figs 2–4). Light microscopy showed that these exposed shrimp had numerous tetrahedral, crystalloid inclusion bodies in nuclei of hepatopancreatic cells.

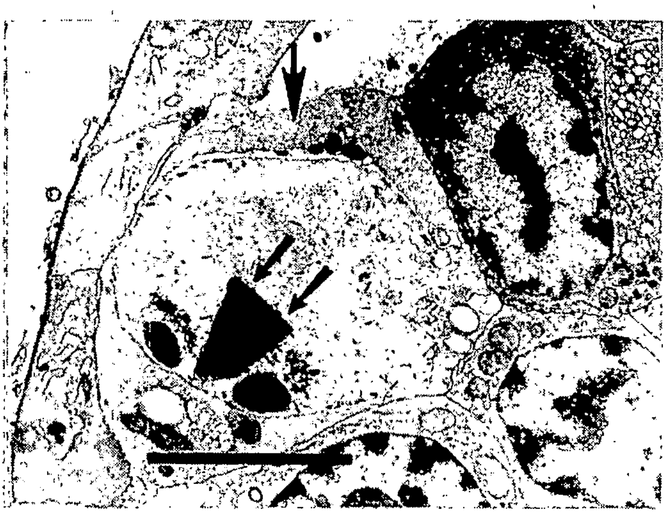

Fig. 3 Low magnification electron micrograph of section through hepatopancreatic tubule from exposed shrimp Note profile of epithelial cell and nucleus (one arrow) containing free virions and intranuclear, crystalline occlusion body (two arrows); note hypertrophy of affected nucleus (bar=5 μm).

A typical free virion within a cell nucleus consisted of a rod-shaped, electron-dense nucleocapsid surrounded by an electron-lucid area' enclosed by one or two envelopes (Figs 2*a*, inset, 2*b* and 5). Nucleocapsids varied in length (from 210 to 316 nm; average 288±31 nm, $n=30$) but had a relatively narrow range of diameters (from 44 to 64 nm; average, 59 nm±6 nm, $n=30$). In cross section, the average diameter of nucleocapsids plus their containing envelopes was 75 nm±7.5 nm ($n=30$). The envelopes surrounding the nucleocapsids (Fig. 5) seemed to be membranous with spatial and structural relationship to the nucleocapsid similar to that of envelopes in nuclear polyhedrosis virus (a *Baculovirus*) from *Bombyx mori*[9].

Virions were distributed in most nuclei in greater numbers near the inner nuclear membrane (Fig. 2*a* and *b*); however, they were associated with or occluded in crystalline bodies at the ultrastructural level in some nuclei (Figs 3 and 4). These crystalline, occlusion bodies were always triangular in section, paracrystalline in structure, and were the only structures observed with the electron microscope that could be related to the intranuclear, tetrahedral, crystalloid bodies observed by light microscopy (Fig. 1). The occluded virions were similar in dimensions and structure to the non-occluded virions.

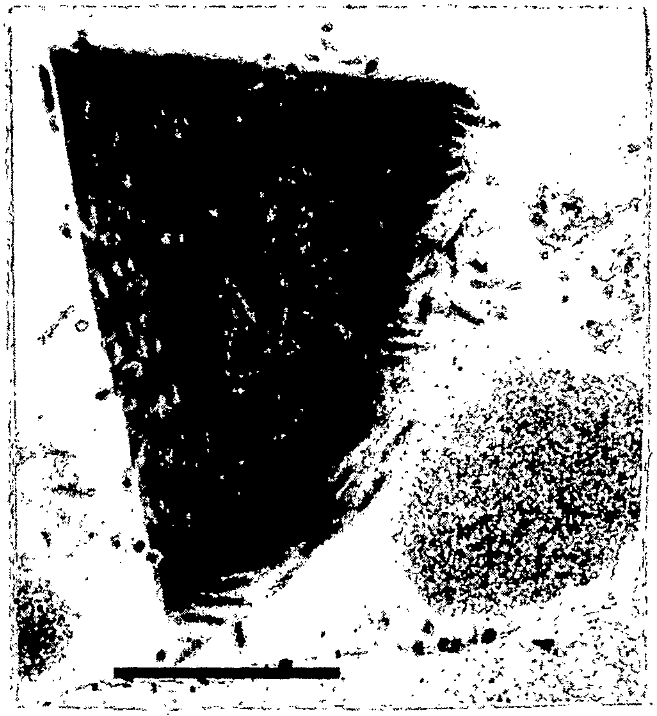

Fig. 4 Higher magnification (EM) of occlusion body from Fig. 3. Note virions occluded randomly within body. This body has a two-dimensional triangular form of crystalloid inclusion body observed with light microscopy in nuclei of hepatopancreatic cells; compare with Fig. 1 (bar=1 μm).

Evidence suggesting early stages of virion formation was found in altered nuclei (20% of cell profiles from exposed shrimp) that contained few or no completely formed virions. U-shaped membranous arrays occurred in these hypertrophied nuclei (Fig. 2*a*). These arrays and associated immature virions were similar to certain stages in early virogenesis of *Rhabdionvirus oryctes* in the rhinoceros beetle[7], and of the virus reported from the whirligig beetle[6].

Ultrastructural cytopathic alterations were associated with the presence of virions. Nuclei that contained virions in large numbers were often 1.5–2 times greater (hypertrophied) in profile area than normal nuclei (Fig. 3). Their cytoplasms, however, usually exhibited no parallel difference in profile area. Heavily affected nuclei had a distinct loss of heterochromatin (Fig. 2*a* and *b*), and some affected nuclei had no heterochromatin. Nucleolar degeneration was evident in nuclei that contained many virions (Fig. 2*b*). The most remarkable change associated with the presence of large numbers of virions was a proliferation of nuclear membranes (Fig. 2*a* and *b*), and their eruption into the cytoplasm to form a multimembranous labyrinth (Fig. 2*b*). Cells whose nuclei contained many virions possessed cytoplasms filled with ribosomes, but without normal numbers of mitochondria or endoplasmic reticulum vesicles (Fig. 2*a* and *b*). Most of these changes have been observed in cells of insects that were infected by *Baculovirus*. At present it is not known if exposure of infected shrimp to PCB enhances the cytopathic effects of the virus.

The following findings suggest a close relationship of the shrimp virus to the *Baculovirus* group[10] of invertebrate viruses. The virions were found in nuclei of hepatopancreatic cells of an arthropod (these cells are analogous in many aspects to fat body and midgut cells of insects). They are rod shaped (bacilliform), enclosed by one to two envelopes or capsids, found both free in nuclei and occluded in crystalline bodies within nuclei[10], and were 288 nm long by 75 nm in diameter, thus falling within the size range of *Baculovirus*[9,10]. Stromata which seemed to be virogenic were present in affected nuclei, and cytopathic alterations of affected cells and nuclei were similar to those reported for *Baculovirus* infections in insects.

In summary, a rod-shaped, free and occluded virus exists in a marine shrimp, indicating that marine crustacea are potential hosts for viruses similar to certain viruses infecting insects and mites. So far, the virus has been found only in shrimp taken from near Cedar Key and experimentally exposed to the toxic chemical, Aroclor 1254 (PCB). The virus probably is a natural parasite, however, previously undetected, of estuarine and marine shrimp.

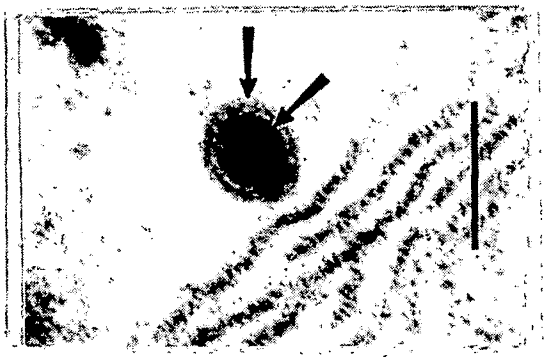

Fig. 5 Cross section of free virion in nucleus near abnormal, multimembranous (six membranes) nuclear envelope; note outer envelope and capsid of the nucleocapsid of virion (arrows) (bar=0.1 μm).

Studies of possible interactions of the PCB and virus in pink shrimp may provide valuable information needed to clarify the relationship between natural infectious diseases and pollutant chemicals in the aquatic environment.

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Inhibitory Effects of Individual Components of the Sex Pheromone of a Tortricid Moth on the Sexual Stimulation of Males

RECENTLY, *cis*-9 and *cis*-11-tetradecen-1-ol acetates, the principal components of the sex pheromone of the smaller tea tortrix, *Adoxophyes fasciata*, and the summerfruit tortrix, *A. orana*, were isolated from virgin females, identified and synthesised[1–3]. Individually, neither component was active even when presented in unusually large amounts. Furthermore, most male moths failed to respond when the two compounds were presented in sequence within a few seconds of each other. In contrast, a mixture of the two compounds at the 10^{-5} μg level elicited the characteristic sexual response from the male moths. Some studies on sex pheromone perception have revealed that geometrical isomers strongly inhibit the response of male moths to their sex pheromone[4–7]. These facts prompted us to examine the effects of individual compounds and their geometrical isomers on the sexual behaviour of the smaller tea tortrix. We found that the individual pheromone components, but not geometrical isomers, had an inhibitory effect on male stimulation by the sex pheromone.

After synthesis, *cis*-9 and *cis*-11-tetradecen-1-ol acetates, and their geometrical isomers, were purified by thin-layer chromatography on $AgNO_3$-impregnated silicic acid and by gas chromatography on 15%-PEGA on Chromosorb W AW. The bioassay procedure was based on that of Tamaki *et al.*[8], in which the mating dance of male moths was used as the criterion of response. The two compounds were assayed individually and in equimolar mixtures by applying them to pieces of filter paper and then exposing ten male moths of the smaller tea tortrix in 200-ml flasks. The quantities of each compound applied varied from 5×10^{-5} to 5×10^{-2} μg. A minimum of four replications was used for each test.

Results are summarised in Table 1. Mixtures of the two compounds evoked a potent response of the male moths ranging from 74% at the lowest concentration tested (5×10^{-5} μg of each compound) to 90% at the highest level tested (5×10^{-2} μg of each compound). When presented individually, however, neither compound elicited the mating dance. *cis*-11-tetradecen-1-ol acetate presented 1 min after males had been exposed to the *cis*-9-isomer also failed to evoke more than a 4% response. Surprisingly, the mixture of *cis*-9 and *cis*-11-isomers also evoked no response when presented 1 min after previous exposure to more than 5×10^{-4} μg of individual isomers. This mixture did elicit a potent response, however, from a group of male moths that had not been previously exposed to the individual compounds. In contrast, previous exposure to the geometrical isomers, *trans*-9 and *trans*-11-tetradecen-1-ol acetates, in concentrations equivalent to the pheromonal components, had no inhibitory effect on the sexual stimulation of males when exposed to the active pheromone.

Clearly the individual sex pheromonal components inhibit the subsequent stimulatory response of male moths of the smaller tea tortrix to the sex pheromone. The inhibitory effect of each pheromone component was apparent when there was an interval of only a few seconds between pre-exposure to one component and subsequent exposure to the mixture. The inhibitory effect was also confirmed by using virgin females. Suppression of mating was 90% and 60% in males pre-exposed for 1 min in 200-ml flasks to 10^{-2} μg of *cis*-9-isomer and *cis*-11-isomer, respectively. The inhibitory effect of individual components of the sex pheromone on sexual behaviour of males of the smaller tea tortrix seems to be involved in the isolation of the smaller tea tortrix from any sympatric species that uses either *cis*-9 or *cis*-11-tetradecen-1-ol acetate as the sex pheromone. Although the olfactory

Table 1 Inhibitory Effect of Individual Pheromonal Components on the Sexual Stimulation of Male Moths of the Smaller Tea Tortrix *Adoxophyes fasciata*

Exposure stimulus*		Amount of each compound presented			
First exposure	Second exposure	5×10^{-5} µg	5×10^{-4} µg	5×10^{-3} µg	5×10^{-2} µg
		Percentage of male response†			
cis-9-TDA plus *cis*-11-TDA	None	74	82	80	90
cis-9-TDA	None	0	0	0	0
cis-11-TDA	None	0	0	0	0
cis-9-TDA	*cis*-11-TDA	2	4	0	2
cis-11-TDA	*cis*-9-TDA	24	4	4	0
cis-9-TDA	*cis*-9-TDA plus *cis*-11-TDA	5	0	0	—
cis-11-TDA	*cis*-11-TDA plus *cis*-9-TDA	20	0	0	—

* Duration of the first stimulus is one minute. TDA shows tetradecen-1-ol acetate.

† Criterion of male response is the 'mating dance'.

mechanisms of the moth are not yet understood, it seems that the male moths possess a complex mechanism that needs simultaneous receipt of the two components of the sex pheromone for release of subsequent sexual behaviour.

The present finding suggests a new approach for controlling the smaller tea tortrix by using only one component of the sex pheromone.

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Genetically Based Behaviour Patterns in *Drosophila melanogaster*

Some time ago Bastock[1] demonstrated that yellow (*y*) mutant males of *Drosophila melanogaster* had reduced success in fertilising normal females. This tendency of females to reject *y* males provides a means by which variation in behaviour between populations might be easily studied.

The studies reported here involve four populations of *Drosophila*. A laboratory wild type (+), a *y* mutant strain, both obtained from Turtox, and two wild type strains (No. 6 and No. 32) collected locally. The last two were selected from a larger collection of eight local populations, preliminary studies of which indicated a considerable variation in the rejection of *y* males by females. The four populations were studied with respect to the extent to which females rejected *y* males. The study was extended in an attempt to select a strain of females which would show a preference for *y* males. We are motivated by a general interest in the examination of genetically based behavioural differences between populations, and the extent to which such differences may be manipulated by selection.

The experiments described are based on the following technique. A single adult virgin female, that had been kept for 2 or 3 d in a small tube with medium, was added, unetherised, to a vial containing medium and in which two males had been present for a similar period of time. Except where otherwise noted, one male was obtained from the same population as the female, and the other from the *y* mutant population.

Table 1 Choice of Males by Females

Female	Males	Success to *y* male	Success to other male	Unknown
No. 6	6, *y*	9	48	8
No. 32	32, *y*	6	137	21
+	+, *y*	3	43	19
y	+, *y*	164	208	18

Comparisons between the populations are shown in Table 1. The experiments were carried out at 25° C, and matings were observed for a period of 2 h from the time of the introduction of the female. Any matings not completed by that time are classed as 'unknown'. Significant differences with respect to the rejection of the *y* males exist between the two local populations, collected from areas approximately three miles apart ($P<0.01$). The *y* females show the highest acceptance of *y* males. Clearly, however, no preference for *y* males exists in any of the populations.

Selection for enhanced acceptance of the *y* males was carried out as follows. Virgin adult female progeny were taken from one or more successful matings to a *y* male in the above experiments, and presented to a pair of males similar to those in the initial experiments. Thus both males are from the initial populations, while the female is from a cross between members of these populations. The next generation of females is again selected from successful crosses to *y*, and these females are again presented to males from the initial populations. Thus a No. 6 female mating with a *y* male gave rise to 6–1 females, and 6–1 females mating with *y* males gave rise to 6–2 females. Either one or two families were used for each generation; thus in each of the first two selected generations one family was used, while in generation 3, for strain 6, two families were used (6–3–1 and 6–3–2). 6–3–1 is derived from one of the two successes to *y* in the previous generation, and 6–3–2 from the other. Selection was only for females; the males were always obtained from the unselected parental populations. Results of the selection experiments for populations No. 6 and No. 32 are shown in Table 2.

It can be seen that no significant differences exist between the data for the first four generations for population No. 6; in fact the data do not differ significantly from that presented for No. 6 in Table 1. A marked change can be seen in the fifth generation where a preference for the *y* male can be seen; this preference is again seen in the sixth and seventh generations. The rareness of successful matings to *y* by No. 32 females accounts for the fewer generations with respect to this population; the data suggest that this population may be less amenable to selection for successful matings with *y* males.

Table 2 Selection for Enhanced Preference for *y* Males

Female and generation	Males	Success to *y* male	Success to other male	Unknown
6–1	6, *y*	4	7	0
6–2	6, *y*	2	35	5
6–3-1	6, *y*	8	25	5
6–3–2	6, *y*	5	15	3
6–4	6, *y*	5	40	4
6–5–1	6, *y*	25	11	11
6–5–2	6, *y*	34	8	9
6–6–1	6, *y*	12	7	7
6–6–2	6, *y*	14	8	2
6–7–1	6, *y*	13	7	3
6–7–2	6, *y*	16	7	5
32–1	32, *y*	2	40	10
32–3	32, *y*	3	21	6
32–3	32, *y*	11	15	4
32–4	32, *y*	12	31	1
32–5–1	32, *y*	6	10	16
32–5–2	32, *y*	8	10	10

It should be realised that females of generations 6–5, 6–6 and 6–7 must contain large amounts of the *y* strain genotype, indeed parts of the *y* strain genotype must be present in all the selected females. Additional experiments in which the original pure *y* females are provided with a choice of No. 6 or *y* males show that the genotype of the pure *y* strain cannot alone account for the results in generations 6–5, 6–6 and 6–7 presented in Table 2. Thus, when the original pure *y* females were provided with a choice of No. 6 males or *y* males, sixty-one chose No. 6 males, twenty-seven chose *y* males and ten were unknown. It can be seen, then, that when pure *y* females are given the choice of No. 6 males or *y* males, the rejection of *y* males is even greater than when their choice is between the laboratory wild type and *y* males (see Table 1). The genetic basis of the preference for *y* males shown by selected females (Table 2) derived from the families 6–5–1,2, 6–6–1,2, and 6–7–1,2 must involve genes from both the original No. 6 strain and from the pure *y* strain, a further understanding of which must await a more detailed genetic analysis.

The results presented here are interesting in a number of respects. At present the simplest way of distinguishing between the two populations, No. 6 and No. 32, is by their behavioural differences described above. Although the *y* mutant is known to be associated with a single gene mutation, the genetic basis of the rejection of *y* males by females is unknown. It is possibly misleading to regard *y* males as offering a low level of stimulus, without carefully defining the mating system. We believe that use can be made of the genetic differences, present in female populations, of preference and rejection for *y* males, shown in the above experiments, in a closer examination of the female responses to male courtship signals.

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Microcapsules as Artificial Food Particles for Aquatic Filter Feeders

IT has long been the aim of the aquaculture industry to replace natural foods with artificial diets which can be manufactured and stored, and there is an obvious need for the development of a successful artificial diet for aquatic filter feeders. Furthermore, if such diets are defined, they can be used to elucidate the exact nutritional requirements of the animals under culture. Provasoli and his co-workers[1,2] have shown that *Artemia* can be reared in a sterile medium, but that a particulate phase is necessary to promote growth beyond the third stage metanauplii.

Artificial food particles are known to be acceptable to a wide range of filter feeders[3,4]. In our experience, food supplied in a particulate form as egg slurry or minced fish[5], or adsorbed on to the surface of particles, invariably leads to bacterial contamination with subsequent deleterious effects. An alternative approach is to use encapsulated diets in which the components are retained within an artificial membrane. The use of microencapsulation techniques in aquaculture has been advocated recently by Meyers and co-workers[6,7] but, until now, no experimental results have been made available. The type of microcapsule that is required depends on the mode of feeding. In the case of molluscan larvae and adult bivalves, which ingest their food whole, the capsule wall should be impermeable and stable in seawater, but readily broken down by a change in *p*H or by the action of digestive enzymes. As far as we are aware, capsules of this type have not been described in the literature. Alternatively, for those larvae or adults which masticate their food, the capsule wall need only be broken mechanically to allow the contents to be ingested.

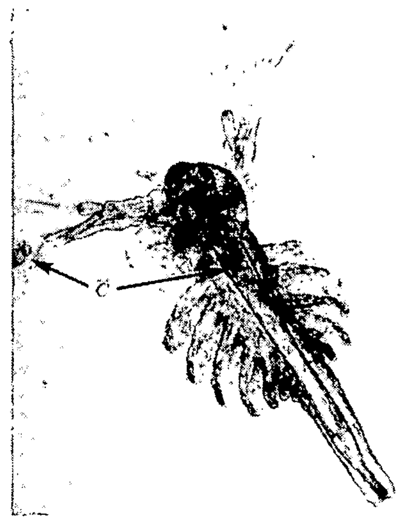

Fig. 1 Microcapsules fed to *Artemia*.

Microcapsules of this second type, with cross linked nylon-protein walls, can be prepared in the laboratory using a modification of the method described by Chang *et al.*[8]. 4,4′-Diamino-2,2′-biphenyldisulphonic acid (6 mmol l^{-1}) is added to give the membrane a resultant negative charge which reduces aggregation of the capsules. The concentration is twice that recommended by Chang *et al.*, to allow for the ionic effects of seawater and to increase the brittleness of the membrane. It was also necessary to double the concentration of sebacoyl chloride (0.036 M) to produce satisfactory capsules in the right size range (20 to 80 μm diameter). The capsule walls are easily ruptured by crustacean mouthparts and a wide range of water-soluble materials and particulate matter can be encapsulated to give almost neutrally buoyant capsules in a size range 5 to 200 μm. The main disadvantage of this type of capsule is that the nylon-protein wall is permeable to small molecules but the capsules can be used to produce defined macromolecular food particles containing starch and protein.

Various Crustacea have been observed feeding in suspensions of microcapsules and it has been shown that all the animals studied, *Macrobrachium* zoea, *Carcinus* zoea, late stage *Balanus* and *Elminius* nauplii, adult *Temora*, and *Artemia*, can rupture the capsule walls and ingest the contents, sometimes together with the crushed outer membrane. Figure 2 shows that, after 4 d, the percentage survival of *Artemia* fed on microcapsules containing 5% starch and 10% haemoglobin exceeded that of the starved controls. After 10 d, 25% of the fed *Artemia* had survived whereas all the starved animals had died. To obtain a mean value for 'growth' within the population of *Artemia*, we have used the growth index of Provasoli *et al.*[2]. This is an arbitrary value related to size and the moult stage reached. The base line is provided by the starved controls. Figure 2 shows that *Artemia* will not progress beyond the third metanauplius stage (growth index 4) without food[1]. Those animals fed on microcapsules, and which survived, reached a growth index of 11 after 34 d with an average development to the juvenile stage although a few individuals reached the adult stage. Clearly, growth and survival was less than that achieved with *Isochrysis* but the encapsulated diet was incomplete and the starch: protein ratio was not optimal for growth of *Artemia*[2].

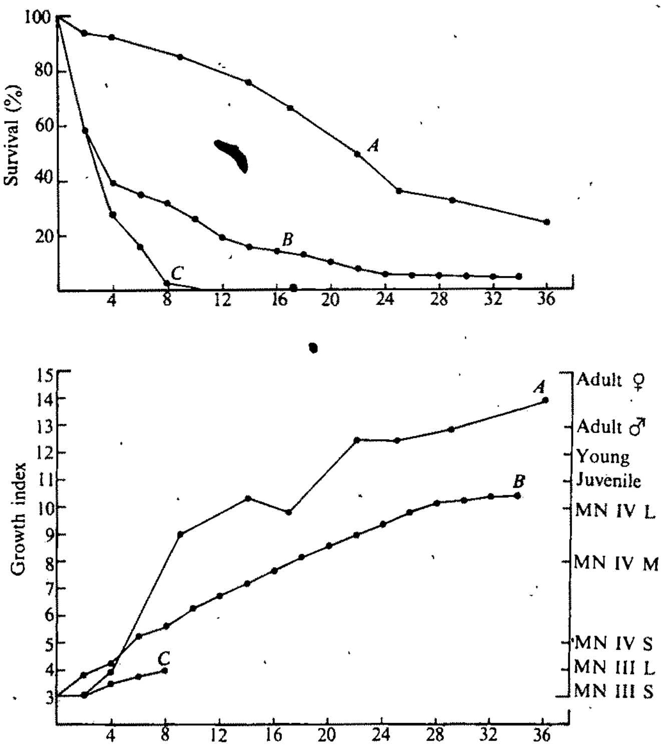

Fig. 2 Survival and growth of *Artemia*. Growth experiments were carried out at room temperature in beakers containing 600 ml of aerated seawater and 120 to 180 *Artemia*. The results are given as the means of two or three replicated experiments, *B* and *C*, and of a single experiment, *A*, with *Isochrysis galbana*. Microcapsules contained 5% soluble starch and 10% haemoglobin. The concentration of food particles was kept in excess of the daily intake for *Artemia* and the seawater and food changed each time the animals were counted. Penicillin G and streptomycin sulphate were added to the cultures at concentrations of 30 mg l^{-1} and 50 mg l^{-1} respectively. *A*, *Isochrysis*; *B*, microcapsules; *C*, starved controls.

A further experiment was carried out to show that U-^{14}C-labelled starch, contained in microcapsules, is assimilated by *Artemia* and the glucose carbon is incorporated into amino acid metabolism. Previous work with Crustacea[9] has shown that carbon from U-^{14}C-glucose is readily incorporated into alanine, glutamic acid, aspartic acid and glycine. Approximately 200 late metanauplius to young adult *Artemia* were fed with microcapsules containing 5% starch (1 μCi mg^{-1}) and 10% haemoglobin for 48 h at 18° C in a litre of seawater. At the end of the feeding period the *Artemia* were removed and washed with isotonic ammonium formate solution, freeze dried and powdered in a micro ball mill. A sample of the dried *Artemia* was extracted with 5% trichloroacetic acid (TCA) and the insoluble protein residue oxidised with performic acid as described by Hirs[10], and hydrolysed with 6 N HCl. The constituent amino acids were separated by one-dimensional thin-layer ion-exchange chromatography[11] and made visible by spraying with a ninhydrin reagent. Radioactivity was determined by scanning the TLC plate using a Berthold Thin-layer Scanner II with a slit width of 1 × 16 mm.

Figure 3 shows that glucose carbon from encapsulated U-^{14}C starch is assimilated by *Artemia* and incorporated into the non-essential amino acids alanine, glutamic acid, aspartic acid and glycine resulting in ^{14}C-labelled protein. No attempt was made to calculate the radiochemical recovery in the protein fraction except to note that the activity was low with a maximum count rate only twice the background count. A further sample of *Artemia* was extracted with chloroform-methanol and the neutral lipid classes separated by TLC on silica gel. Traces of ^{14}C-label were detected in the fatty acid component.

It has been our intention to show that microencapsulation represents a feasible approach towards the development of artificial diets for filter feeders. Animals with very different food preferences, ranging from *Artemia* cultured on phytoplankton, to *Macrobrachium* zoea which feed on *Artemia* itself, were observed to accept and ingest the contents of microcapsules provided these were supplied in the correct size range, 20 to 250 μm. Although the ideal capsule should have an impermeable membrane which could either be ruptured mechanically or broken down by digestive activity, much can still be accomplished with the present capsules despite their permeability. Capsules containing starch and protein could be used as artificial food particles, together with natural food organisms, to provide essential nutrients such as vitamins. Lipids might also be adsorbed on to starch particles and incorporated into the capsules, which could then be used to determine the optimum starch: protein: lipid ratios for filter feeders. Metabolic studies in which ^{14}C-labelled starch (or protein) is incorporated into capsules are already possible. Labelled fatty acids could be adsorbed on to starch particles as described by Morris *et al.*[12] and encapsulated for filter feeders. In addition, other metabolites or foreign compounds could be fed in an encapsulated form either adsorbed or attached covalently to a suitable high molecular weight carrier molecule.

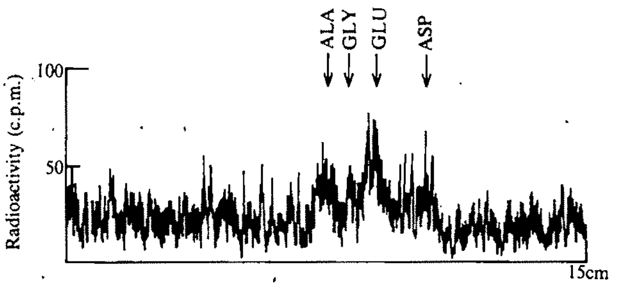

Fig. 3 Separation of constituent amino acids from protein hydrolysate of *Artemia*. Precoated Ionex-25 SA, Na^+ form TLC plate (Macherey, Nagel and Co.) without pre-equilibration. Elution buffer, *p*H 3.3, sodium citrate, 0.4 N Na^+, 0.4 M citrate at 50° C. Approximately half the total protein hydrolysate from 2 mg freeze-dried *Artemia* was applied to the plate as a 1 cm band and eluted to a distance of 15 cm. Individual amino acids were identified by comparison with a mixture of known standards.

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Degradation of *p,p'*-DDT in Reducing Environments

p,p'-DDD (*p,p'*-TDE; 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethane) is an extremely persistent environmental contaminant[1]; it is a well known insecticide but is also derived from *p,p'*-DDT (1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane) in the environment by a process of reductive dechlorination. The conversion of DDT to DDD has been observed in a wide variety of biological systems, both living and dead[2]. The mechanism by which this degradation of a highly chlorinated molecule takes place in the environment is of considerable interest but has not been clearly established[3,4].

We have recently re-examined the environmental degradation of DDT and have measured DDD/DDT ratios after exposing DDT to a variety of conditions: sterile and non-sterile; oxidising and reducing; and appropriate combinations. The systems examined were: (1) sewage sludge, (2) baker's yeast, (3) iron salts and iron porphyrins, (4) bacterial cultures and (5) annelid worms. The results from this set of conditions, which ranged over complex biological situations, pure cultures and chemical systems, have led us to the conclusion that the conversion of DDT to DDD in the environment is mediated by reduced iron porphyrins and is not an essential part of cell metabolism.

Secondary sewage sludge converts DDT to DDD under anaerobic conditions[5-8], with a half-life of as little at 3 h, but DDD is itself stable. The data in Table 1 show that not only is this ability not destroyed by sterilisation with γ-radiation (Experiments 1 and 5) but that even after inactivation by aeration (Experiment 6) or by autoclaving[5] (Experiment 3), activity can be restored instantly if the *Eh* is sufficiently lowered by addition of sodium dithionite (Experiments 4 and 7), a reducing agent widely used in biochemical studies[9]. This suggests to us that direct microbial metabolism does not account for the reductive dechlorination of DDT in untreated sewage sludge.

Table 1 Degradation of *p,p'*-DDT in Anaerobic Sewage Sludge

Exp. No.	Sterilisation	Reduction or oxidation	*Eh* (mV)	DDD/ DDT	% ^{14}C extracted
1	—	—	−285	0.8	71
2	—	Dithionite	−660	2.0	63
3	Autoclaved	—	−210	0.1	84
4	Autoclaved	Dithionite	−640	2.3	76
5	5 Mrad γ	—	−220	1.0	56
6	—	Aerated	+50	0.1	71
7	—	Aerated, then dithionite	−645	1.7	57

Aliquots (10 ml) of anaerobic sewage sludge were sterilised (15 pounds per square inch, 15 min or ^{60}Co source at 0.45 Mrad h^{-1}) and reduced (50 mg sodium dithionite in 0.5 ml water) or oxidised (rapid aeration for 90 min at 20° C) as indicated. ^{14}C-*p,p'*-DDT (107 μg, 0.45 μCi) in ethanol (0.1 ml) was added to each sample and the mixtures incubated (37° C, 90 min). The sludge was obtained from the anaerobic digestor of the Bristol Corporation Sewage Treatment Works, and was concentrated by decantation to 5% dry weight solids. ^{14}C-*p,p'*-DDT (98% pure) was obtained from the Radiochemical Centre, Amersham, and unlabelled *p,p'*-DDT from Aldrich (99% pure). *Eh* measurements were made with a platinum spear electrode, a calomel reference electrode and a Philip Harris *p*H and Redox meter: readings were taken 1 min after the electrodes were inserted into the sample and the platinum spear was flame-cleaned between each determination. The samples were extracted (hexane : isopropanol 1 : 1, 3 × 10 ml), the combined hexane layers were analysed by GLC as previously described[6,8] and by scintillation counting. DDD/DDT ratios are calculated directly from peak heights. Aqueous dithionite alone yielded only a small amount of DDD (see Table 3). Results shown are the mean of duplicate determinations. The DDT and DDD together account for 30–60% of the extracted ^{14}C. More polar products, similar in chromatographic behaviour to those noted by Miskus *et al.*[13], account for the remainder of the label in these and in the relevant experiments quoted in Tables 2 and 3.

A readily available microbial system is baker's yeast in which Kallman and Andrews[10] have reported substantial conversion of DDT to DDD over periods of several days. In our experiments, however (Table 2), no significant reaction took place in fresh yeast suspensions in 1.5 h at 37° C unless the *Eh* was lowered by addition of sodium dithionite. Previous autoclaving brought a marked enhancement of this effect, suggesting that autoclaving released a chemical factor which at the appropriate *Eh* was an effective reducing agent for DDT.

Various putative reductants for the DDT to DDD conversion have been put forward. For example, Glass[11] claimed that a ferrosoferric-ferrous couple was effective at high *p*H, but we achieved only a very slow reaction. Increasing the ferrous ion concentration in sewage sludge did not significantly increase the ability of the sludge to degrade DDT to DDD, nor did it restore this ability to autoclaved sludge. Addition of ethanol to increase the solubility of DDT in the presence of ferrous ions also failed to increase the extent of reaction. Castro[12] reported the rapid oxidation of a reduced iron porphyrin by DDT in isopropanol : acetic acid (1 : 1) saturated with KCl under nitrogen. Later, Miskus *et al.*[13] suggested that reduced porphyrins might be the factor responsible for conversion in biological systems. This attractive suggestion has been reiterated[5,14] and cytochromes

Table 2 Degradation of *p,p'*-DDT Yeast Cell Suspensions

Sterilisation	Reduction	*Eh* (mV)	DDD/ DDT	% ^{14}C extracted
—	—	+165	0.1	91
—	Dithionite	−510	0.4	91
Autoclaved	—	+50	0.1	97
Autoclaved	Dithionite	−600	1.3	91

Aliquots (10 ml) of an aqueous 10% w/v suspension of commercial yeast (Distillers, Yeast and Food Division) were used. The materials and procedure were otherwise the same as described under Table 1.

and other protein-bound haem compounds have since been shown to degrade DDT slowly under reducing conditions[15]. Using haematin dissolved in dilute sodium hydroxide ($pH > 11$) Miskus *et al.*[13] obtained 10% conversion of DDT to DDD in 4 h with sodium dithionite as a reducing agent. Solubility is a problem in conducting experiments with Fe(II) porphyrins in the *p*H range normally associated with biological systems. Haematin (the Fe(III) porphyrin corresponding to the Fe(II) porphyrin, protohaem) is soluble in aqueous 0.1 N Na_2CO_3 but precipitated when it was reduced to protohaem by the addition of sodium dithionite. The addition of a detergent or ethanol prevented this precipitation and the results we thus obtained with protohaem and DDT are shown in Table 3. The conversion of DDT to DDD can therefore be accomplished rapidly and in a catalytic manner by an Fe(II) porphyrin at a *p*H frequently associated with biological systems (Table 3; Experiments 1–3). Rapid degradation of DDT was also obtained when ferrous sulphate was substituted for sodium dithionite (Experiment 4), but no significant degradation was observed with ferrous sulphate under conditions similar except for the absence of haematin. The addition of haematin and detergent to anaerobic sewage sludge greatly increased the DDT to DDD conversion (Experiments 7–10).

Table 3 Reaction of *p,p′*-DDT with Reduced Haematin

Exp. No.	Haematin (mg)	Tween 80 (mg)	Ethanol (ml)	Reducing agent	DDD/DDT	% ^{14}C extracted
1	0.6	—	—	Dithionite	0.25	54
2	0.6	25	—	Dithionite	0.83	42
3	0.6	—	1.0	Dithionite	2.7	84
4	0.6	25	—	Ferrous sulphate	1.9	28
5	—	25	—	Dithionite	0.09	46
6	—	—	1.0	Dithionite	0.06	95
7	—	—	—	Sludge	0.21	96
8	0.3	—	—	Sludge	0.36	85
9	—	200	—	Sludge	0.85	80
10	0.3	200	—	Sludge	3.5	81

DDT (3.55 mg, 0.1 μCi in Experiments 1–6; 1.78 mg, 0.05 μCi in Experiments 7–10) in ethanol (0.2 ml and 0.1 ml respectively) was added to each mixture of haematin (Koch-Light Laboratories), Tween 80 (Hopkin and Williams), ethanol, 0.5 ml 0.1 N aqueous Na_2CO_3 solution, and chemical reductant (25 mg) or sludge (10 ml). The source of the DDT and the incubation and analytical procedures were as described for Table 1, except that in Experiments 1–6 products were extracted into 10 ml ether by very vigorous agitation. The DDT : haematin molar ratio was 10 : 1.

The foregoing chemical experiments demonstrated that DDT to DDD conversion was effected in any situation in which DDT and reduced iron porphyrins were brought together in solution.

Reports have appeared and continue to appear[4,10,16–20] reporting organisms which either do, or do not, readily metabolise DDT but they do not, mostly, purport to show the use of DDT as a sole carbon source. Our next experiment simulated the natural exposure of DDT to porphyrins, or porphyrin-like compounds, contained in microorganisms, at various oxidation/reduction potentials.

Bacteria derived from anaerobic estuarine sediments were grown in a chemostat on a medium containing about 10 p.p.m. DDT. Dilution rates down to 0.25 h^{-1} were used, gassed either with air to maintain aerobiosis or with nitrogen. We failed to observe any degradation of DDT under these conditions of continuous growth. But when the chemostat was turned off, DDT conversion was soon observed in the culuture vessel and also occurred in the effluent reservoir where there was considerable autolysis.

In another experiment, pure cultures of twelve species of bacteria from ten genera were grown aerobically on nutrient agar overnight and collected, and aqueous suspensions containing about 10^9 viable cells ml^{-1} were incubated with 10 p.p.m. DDT, with and without sodium dithionite, at 37° C for 1.5 h. Conversion of DDT was significant only in cell suspensions containing dithionite and was enhanced by using cell suspensions which had been previously heated at 100° C for 10 min.

In a laboratory experiment[21] individuals of *Arenicola marina* (lugworms) living in well-aerated artificial burrows containing 2,000 p.p.m. of DDT were unable to degrade ingested DDT. But 48 h after death, when lysis and decay were well established, about 70% of the incorporated DDT was found to be converted to DDD and other products, confirming similar reports[1–3,14] of degradation by dead animal tissues in anaerobic conditions or where no precautions had been taken against bacterial decay.

We believe that the foregoing rationalisation and extension of existing data bearing on the reductive dechlorination of the normally highly persistent DDT molecule has considerable environmental significance. Most living material contains iron porphyrins bound with protein in complex molecules such as haemoglobin, cytochromes and catalases. Decay should release iron porphyrins[22] which, under the reducing conditions pertaining in the anaerobic environments characteristic of dead and decaying matter, would catalyse rapid conversion of any available DDT to DDD and other products. In terms of the breakdown of the total world mass of DDT this pseudo-biological mechanism could appreciably affect the environmental half-life, since it would come into operation whenever the DDT encountered the appropriate situation in passing through the food web. Anaerobic microenvironments are ubiquitous and include the sulphide biome of reducing sediments[23], the decaying cell contents of dead organisms and the lower portions of the alimentary tract of animals. The operation of such a process should result in high DDD/DDT ratios in the tissues of animals scavenging on dead and decomposing organisms.

Work is in progress to identify the other products formed in the reaction of DDT with reduced iron porphyrins and to seek evidence for the occurrence of this reaction in various environments.

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Freezing resistance in winter flounder *Pseudopleuronectes americanus*

THE blood serum freezing points of most marine teleosts are within the range of −0.5 to −0.8° C[1]. As large regions of the oceans have temperatures as much as 1° C below this range[2], many marine fish must either increase the osmotic concentrations of their blood or exist in a supercooled state. The latter is unstable and in the presence of ice, supercooled fish will freeze and die.

The winter flounder, *Pseudopleuronectes americanus*, inhabits the coastal waters of eastern North America from Virginia to Labrador[3]. In the northern part of their range they occur during the winter in large numbers in ice-covered shallow bays[4]. Using a freezing point osmometer (Fiske) Pearcy[5] found that the blood serum of winter flounder from the Mystic River Estuary, Connecticut froze at −0.63° C during summer and at −1.15° C in winter. NaCl accounted for 83% of the freezing point depression in summer, but only 57% in winter. The solutes responsible for the remainder of the depression in winter fish were not identified. Umminger[6] investigated freezing resistance in winter flounder taken from the same area. Using data obtained with a vapour pressure osmometer he concluded that the serum freezing points of winter fish were not unusually low (−0.71° C).

TABLE 1 Freezing point, melting point and inorganic constituents of whole and dialysed serum from *Pseudopleuronectes americanus*.

Specimen and environmental parameter	Freezing point (° C)	Melting point (° C)	Melting point − freezing point (° C)	Sodium (mM l⁻¹)	Chloride (mM l⁻¹)	% △ due to NaCl
Winter flounder serum March; water − 1.2° C (12)	−1.37 ± 0.31	−0.75 ± 0.03	0.62 ± 0.35	250 ± 12	178 ± 6	58.1
Winter flounder serum August; water + 17° C (8)	−0.69 ± 0.07	−0.67 ± 0.07	0.02 ± 0.001	194 ± 6	147 ± 14	94.0
Winter flounder March; water − 1.2° C						
Dialysed serum	−0.65	−0.01	0.64	—	0.0	
Dialysate	−0.72	−0.70	0.02	—	172	

During the winters of 1972 and 1973 we investigated the freezing resistance of winter flounder collected by trawl at depths of 3 to 10 m in Head Harbor of St Margaret's Bay, Nova Scotia. Specimens were collected in March, a few days after the ice had begun to break up, when the water temperature was −1.2° C. The lower incipient lethal temperature (freezing temperature) determined by recording the lowest temperature at which the flounder could survive in the presence of ice was −1.4 ± 0.1° C.

In the serum of the Antarctic fish, *Trematomous borchgrevinki*, there is a group of organic 'antifreeze' compounds (glycoproteins) responsible for the serum's unusually low freezing point[7]. These glycoproteins lower the freezing point of water without affecting the melting point by coating the surface of ice crystals, thereby removing them as possible nucleation sites[8,9].

To determine whether freezing resistance in the winter flounder is based on a similar mechanism, the freezing-melting behaviour of their serum was studied.

The freezing and melting points of serum samples were determined by placing the serum in small capillary tubes which were sealed at one end and plugged with mineral oil at the other. A small seed crystal was formed in the sample by spray freezing and the sample immersed in a refrigerated viewing chamber. The temperature of the chamber was controlled to within ±0.01° C and the seed crystal (0.25 mm diameter) viewed through a microscope. The temperature of the bath was raised or lowered 0.02° C per 5 min. The temperature at which the seed crystal disappeared was taken as the melting point and that at which the crystal grew as the freezing point. For an NaCl solution, the temperatures of melting and freezing are within 0.02° C of each other. For the serum of winter flounder collected in March, however, the freezing and melting points are widely separated (Table 1). This difference has been termed thermal hysteresis. Serum sodium and chloride ion concentrations accounted for 58% of the serum freezing point depression (−1.37° C) of March flounder and 94% for serum from fish collected in August when the water temperature was 17° C (serum freezing point = −0.69° C).

Dialysis showed that the major portion of the freezing point depression not due to NaCl in March fish was associated with the colloidal fraction of the serum. After exhaustive dialysis (tubing of molecular weight cutoff 3,500 daltons) against distilled water both the dialysed serum and the dialysate were returned to the original volume. Table 1 shows that the solutes responsible for the thermal hysteresis were unable to pass through the dialysis tubing and must therefore have molecular weights greater than 3,500 daltons.

These antifreeze compounds were stable at a temperature of 100° C and soluble in 10% trichloroacetic acid (TCA). Therefore, a crude preparation of the antifreeze was made by dialysing the serum and boiling it for 10 min followed by centrifugation at 15,000*g* for 20 min. The supernatant was diluted (1:10) with TCA and again centrifuged. After the TCA was removed from the supernatant by dialysis, lyophilisation resulted in a fluffy white residue. The freezing and melting points of aqueous solutions of this antifreeze preparation were determined at several concentrations (Fig. 1). The difference in freezing and melting points varied with concentration.

The discovery of thermal hysteresis in winter flounder serum shows that the lowering of the freezing point of water by the antifreeze is not a colligative effect. In view of this, the freezing points reported by Umminger[6], which were derived from vapour pressure measurements, would not be expected to agree with those reported by Pearcy[5] as the vapour pressure of solutions is a manifestation of the number of dissolved particles. The serum freezing points (−0.71° C) observed by Umminger do, in fact, agree with the melting points we have obtained (Table 1). Freezing points measured with a freezing point osmometer are in reasonable agreement with those determined with the capillary technique. It is clear that in studies of freezing resistance, careful consideration should be given to choosing a method for determination of freezing points. Is seems that visual examination of crystal growth under a carefully controlled temperature regime is the best method and is biologically the most meaningful because it agrees with the organismal freezing point.

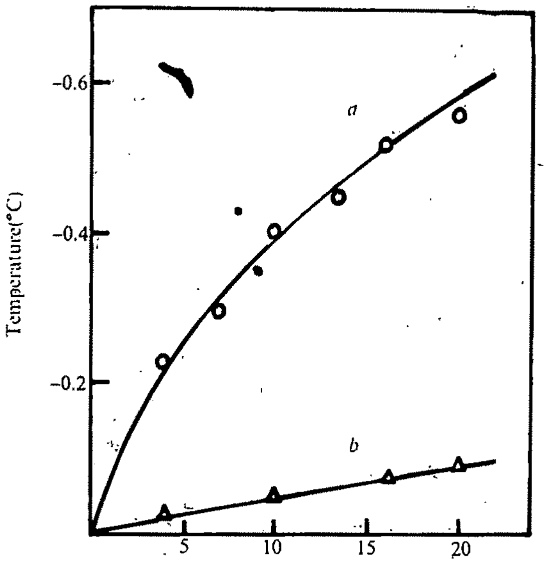

FIG. 1 Comparison of the freezing and melting points of aqueous solutions of antifreeze isolated from the blood serum of *Pseudopleuronectes americanus*. o, Temperatures of ice crystal growth in the presence of a seed crystal (freezing point); △, melting points of the seed crystal.

The freezing behaviour of the serum and partially purified antifreeze solutions was also studied by the use of freezing curves. The freezing points obtained with this technique agree with those obtained from the capillary method (Fig. 2 and Table 1). The shape of the freezing curve of the serum of the Canadian plaice, *Hippoglossoides plattessoides*, which does not produce an organic antifreeze, resembles that of NaCl because the principal solute responsible for the depression of the freezing point is NaCl. The shape of the curve of NaCl and the plaice serum results from the fact that as freezing proceeds pure water freezes out leaving behind a more concentrated salt solution with a lower freezing point. Thus, during freezing the temperature of the sample remains slightly warmer than the bath temperature until all the solvent freezes—at which time the sample temperature rapidly approaches that of the bath. The shape of the freezing curve of the serum of the flounder differs from that of the plaice by the presence of a slight flat region near the freezing point. The flatness is more pronounced in the freezing curve of a solution of flounder antifreeze and much more so in a solution of the glycoprotein antifreeze from the Antarctic fish. The long plateau region of the latter results from incorporation of the glycoprotein into the solid phase during freezing. The glycoproteins are equally partitioned between the liquid and solid phases which indicates they depress the freezing point of water by coating the surface of seed ice crystals, thereby preventing water molecules from joining the ice lattice[7–9]. Although no measurements have been made to show that the flounder antifreeze is equally partitioned between the liquid and solid phases during freezing, the flatness of its freezing curve strongly suggests that this is the case.

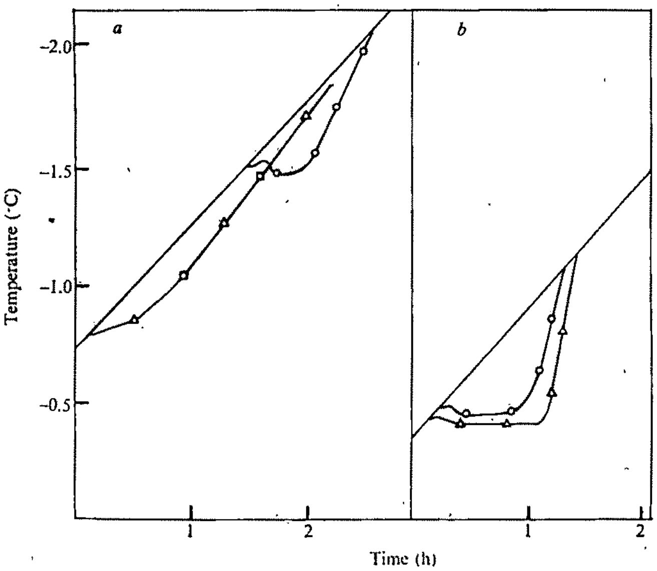

FIG. 2 *a*, Freezing curves of an aqueous 220 mM NaCl solution (△); of the blood serum of the Canadian plaice, *H. Plattessoides* (□); and winter flounder, *P. americanus* (o). *b*; Freezing curve of a solution of antifreeze (10 mg ml⁻¹) isolated from the serum of *P. americanus* (o); and of a solution of antifreeze (10 mg ml⁻¹) isolated from the blood serum of the antarctic fish *Trematomus borchgrevinki* (△). The freezing point obtained from a freezing curve is the temperature where the sample temperature deviates from the bath temperature—indicating a warming of the sample resulting from the latent heat of fusion.

The thermal hysteresis and the freezing curve data lead to the inference that the mechanism of action of the flounder antifreeze is similar to that of the glycoproteins in Antarctic fish. Acrylamide gel electrophoresis and amino acid analysis indicate that the antifreezes of the two fishes differ markedly in composition. Therefore, it seems that distantly related families of cold water fishes have adapted to freezing conditions by evolving different large molecular weight compounds which, however, possess similar antifreeze activity.

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book reviews

Loving only the dead

Ten Years after Ivan Denisovich. By Zhores A. Medvedev. Translated from the Russian by Hilary Sternberg. Pp. xiv+202. (Macmillan: London and Basingstoke, November 1973.) £3.95.

THIS severely factual book, by a distinguished Soviet biologist temporarily working in England, encompasses three themes: the official persecution of Alexander Isayevich Solzhenitsyn; the elevation of the Big Lie to an instrument of policy; the ability of a profound creative writer who is also a fearless patriot to console the wounded spirit of his fellow citizens, oppressed not only by arbitrary cruelty but also by Universal Silence. For the reader in the West, Solzhenitsyn's novels are documentary accounts of reality distilled through the writer's imagination, and thereby an aesthetic experience: for the Soviet reader, they are the healing voice of truth, of great evil admitted, faced and endured.

Medvedev is a 'dissident' who has deserved well of his fellow Soviet citizens by disseminating, in *samizdat*, his detailed account of the official suppression of scientific genetics in the Soviet Union at the behest of the vicious charlatan, Lysenko. Solzhenitsyn wrote to Medvedev to encourage him when he was attacked for his factual writings about Lysenko, and their friendship stemmed from this act of support. The book under review makes it very clear how vital such acts of support by compatriots are to a Soviet citizen who is at odds with the authorities, and his recognition raises difficult questions for us in the West, who must decide whether our words of support are a help or a peril.

In his new book, Medvedev describes the circumstances of the publication in 1962 of *One Day in the Life of Ivan Denisovich* (on the express authority of Khrushchev who was then still concerned with surreptitiously blackening Stalin's memory); the first signs of official disapproval, when Solzhenitsyn was passed over for the Lenin Prize and had the manuscript of *The First Circle* and other papers confiscated; the prolonged but frustrated attempts by the remarkable literary editor, Tvardovsky, to publish *Cancer Ward* in his journal *Novy Mir*; the attempt to turn Solzhenitsyn into a non-person at the Fourth Writers' Congress; his expulsion from the Writers' Union and that of Tvardovsky from his editorship; the prolonged official chicaneries in connection with Solzhenitsyn's Nobel Prize, which in the event was never presented in person; and, running like a refrain through the whole book, the many attempts by officialdom to blacken Solzhenitsyn's character at home and abroad by Big Lies about his wartime record, social background, daily habits, alleged support of Russian émigré organisations, by untruthful articles about his unpublished confiscated novels, unauthorised publication of a juvenilium disowned by Solzhenitsyn, and so on. Apart from that, Medvedev also quotes numerous specific instances of Solzhenitsyn's friends and admirers who lost their membership in the Party or Writers' Union, or their jobs, for either supporting Solzhenitsyn or for opposing or traducing him when told to do so by authority.

All this is presented in Medvedev's characteristic style of dry and scrupulous precision. (Readers who recall the detailed circumstantial evidence for postal censorship in his earlier book, *The Medvedev Papers*, will recognise the style.) A whole chapter is here devoted to the account of a determined detective exercise which eventually proved that mendacious articles about Solzhenitsyn published in two obscure periodicals in Italy and Poland had in fact originated in Moscow. Medvedev throughout evinces two predominant concerns—for accuracy and for the law. This is evident from the first page: the preface explains the circumstances in which this book was published, connected with Medvedev's concern not to offend against the new restrictive Soviet laws on publication abroad by Soviet authors, consequent on the Soviet Union's signature of the International Copyright Convention. In many places in the book, he explains in detail why various official acts against Solzhenitsyn were not only oppressive but also illegal. It seems that the dread censorship itself, Glavlit, is strictly illegal. It is impressive and indeed encouraging that Soviet 'dissidents', Medvedev notable among them, and unlike so many dissidents in the West, observe their country's laws more scrupulously than their own government. When, one distant day, the laws of the Soviet Union become more liberal, one may hope that they will still be obeyed by the citizens and perhaps even by the government.

Medvedev's skills do not include evocative portraiture. Solzhenitsyn and Tvardovsky do indeed come alive in the book, but only through their own deeds and words, carefully related; Medvedev does not attempt set descriptions or indeed flights of literary criticism, though he does quote verbatim a few choice pieces of official "criticism". But then, this book is concerned with history, and he leaves literary analysis to others suitably qualified. A number of Russians who befriended Solzhenitsyn, such as the physicists Kapitsa and Pavlinchuk and the cellist Rostropovich, are briefly sketched, but again their personalities emerge through their reported deeds.

The book quotes the entire text of Solzhenitsyn's message to the Fourth Writers' Congress which he was prevented from attending. This is an eloquent attack on censorship and the personal oppression of individual writers. He points out that once a troublesome writer is dead, eventually his works find their way back to the Soviet public; Pasternak is a case in point. Solzhenitsyn quotes Pushkin with heavy irony: "They are capable of loving only the dead". He might have added the bitter words of Auden: "Let us praise while we can The Vertical man, Though we honour none But the horizontal one". But one must face the question; when we in the West praise the vertical man and condemn his oppressors, those who constantly strive by persecution, imprisonment and starvation to turn him into a horizontal one—do we help or do we hinder, do we protect or do we endanger? Understandably, Medvedev does not address himself to this question, and during a recent televised discussion when this question was tangentially debated, he maintained a dignified silence. Let us see what advice Solzhenitsyn himself has for us.

In the *New York Review of Books* of October 4, 1973, there is a transcript of a recent interview granted by Solzhenitsyn. He has this to say: "It must be understood that the East is not at all indifferent to protests from Western society. On the contrary it mortally fears them—and only them; but only when such protest is expressed by the strong and unified voices of hundreds of eminent people, by the opinion of an entire continent. Only then can an 'advanced' regime be shaken. But when protests are timid and isolated, made without faith in their success and accompanied by the inevitable reservation that 'it is also the same in Greece, Turkey and Spain', they provoke only laughter from our oppressors. . . . The West by its publicity has already done much to help and save many of our oppressed . . .". And the first of the modern dissidents, Pavel Litvinov and Larissa Daniel, in 1968 explicitly addressed their particular protest to world public opinion, adding that to address their own government and news media would be useless.

Such a message presupposes a response. And on the nature and indeed the wisdom of such a response, opinion in the West is deeply divided. Whether the victims are scientists or ballet dancers who wish to emigrate to Israel, writers who cannot publish at home, musicians who cannot perform abroad, some wish to follow Solzhenitsyn's fierce and explicit call, others prefer private pressure through private channels. Since such pressure must necessarily remain secret, the rest of us cannot form an opinion of its efficacy. Those who are in a position to exert private pressure and prefer it are right to follow this path, so long as they do not seek to warn off the grass others who see hope in a public approach. These still have to make the difficult decision whether or not to mention names, to specify the individual they are seeking to help. One must make one's own decision in each instance: perhaps fame is a form of safety, so that a notable figure will be helped by a protest emanating from a group of other notable figures (as Solzhenitsyn indicates, they need to be eminent), while an unknown victim, if named, may well suffer the more. We cannot know for sure, but when distinguished men like Solzhenitsyn, Sakharov and Levich all offer the same urgent advice, we had better heed it. Some differ: Roy Medvedev, Zhores' brother, has criticised Sakharov and Solzhenitsyn for calling for pressure from the West, and Sakharov in turn has just issued a statement regretting this, and maintaining his advice. Opinions do conflict among the victims, but this does not absolve each of us from the necessity of facing the issue and reaching a decision. Whatever their decisions, everyone with a concern for the future of Soviet society should read Zhores Medvedev's book.

ROBERT CAHN

Human chromosomes

Human Chromosomes. By E. H. R. Ford. Pp. xiii+381. (Academic: New York and London, April 1973.) £7.

DR FORD has written a book on human chromosomes which should be useful for students in the important and expanding field of medical genetics. The first third of the book includes well illustrated descriptions of mitosis and meiosis and of the practice of karyotyping. This is followed by an outline of molecular genetics and of chromosome structure leading to a discussion of dosage compensation and of the origin of chromosomal abnormalities. The last and strongest section discusses in detail the various syndromes associated with chromosomal abnormalities before describing more general matters. These include the fascinating and thought-provoking relationships between fingerprints, mental abnormality and extra chromosomes, and the problem of the relation between chromosomal abnormalities and acquired diseases like cancer.

Anybody writing a textbook in a rapidly developing field must expect to be overtaken by events, but it is particularly unfortunate that the full impact of the fluorescent and Giemsa banding techniques on the identification of human chromosomes has come after most of this book was written. These latter methods are certainly discussed but the largely redundant and inadequate earlier methods of identification are still given equal weight. More seriously many of the ambiguities of chromosome assignment which appear in the later sections could have been removed.

The outline of molecular genetics, which authors of this kind of text nowadays rightly feel they must include, serves at least one useful purpose in that it underlines the complete inadequacy of current thinking on the control of the expression of genes in explaining the necessity for dosage compensation in the X or the far-reaching effects of any particular trisomy. Neither the bacterial repressor mechanism nor even the hypotheses involving chromatin condensation in gene switching lead one to predict that an extra complete chromosome should have such disastrous effects. The problem is challenging; obviously tolerance of simple heterozygosity is essential but does this include control sequences as well as sequences specifying proteins? Tandem doubling of particular loci must also be allowed; otherwise one cannot account for the increase of genetic material on the evolutionary time scale or for the evolution of haemoglobin. In addition the new methods of identification are showing that translocations of visible portions of chromosomes are common, which suggests that the functional relations of large sections at least of chromosomes are not essential. The basic requirements of life and even the simple control of the presence of enzymes are, however, compatible with the wide karyotypic variation common in tissue culture lines. It seems inescapable that it is only the ordered development of a differentiated organism which requires a precise chromosome complement, but how are these chromosomal interactions mediated?

A book like this seems therefore to be essential reading for anybody concerned with gene expression and with the investigation of human chromosomal abnormality and genetic counselling.

P. M. B. WALKER

Variable structure

Theory and Applications of Variable Structure Systems. Edited by R. R. Mohler and A. Ruberti. Pp. x+232. (Academic: New York and London, December 1972.) $8.

"VARIABLE structure" in the title is not closely defined anywhere in the book, but has been interpreted by the different authors to include any system which cannot be described by linear differential (or difference) equations with constant coefficients. This is not an easily defensible terminology, since the structure of systems which cannot be described in this simple way is usually no less fixed than the structure of those which can. Indeed, the most interesting results presented by the authors relate to fixed aspects of structure.

Nevertheless, the motivating idea of the seminar from which the papers are taken is clear. Much work has been done on linear constant systems, and many of their structural properties are known. More general systems can have different structural properties, which may be better adapted to describing a given physical situation. The aim of the seminar was to bring together applied mathematicians interested in generating and applying results for such systems.

The outcome is an interesting collection of papers which give a view of the present state of the subject. As would be expected, most progress has been made where the problems could be most closely restricted. In particular the theory of bilinear systems has now emerged as an incomplete but already well developed area. The work of Ruberti and of Brockett on the theory of these systems is particularly noteworthy, while Mohler lists a wide range of actual or potential applications: these include economic, biological and engineering systems.

Other topics include identification, modelling, model approximation and near-optimal control. Several papers deal with biological or medical applications—models of the brain, prostheses, ecological systems—where linear constant models have limited use. The book will interest a wide range of readers, but much of its content is inaccessible without a good mathematical background.

H. H. ROSENBROCK

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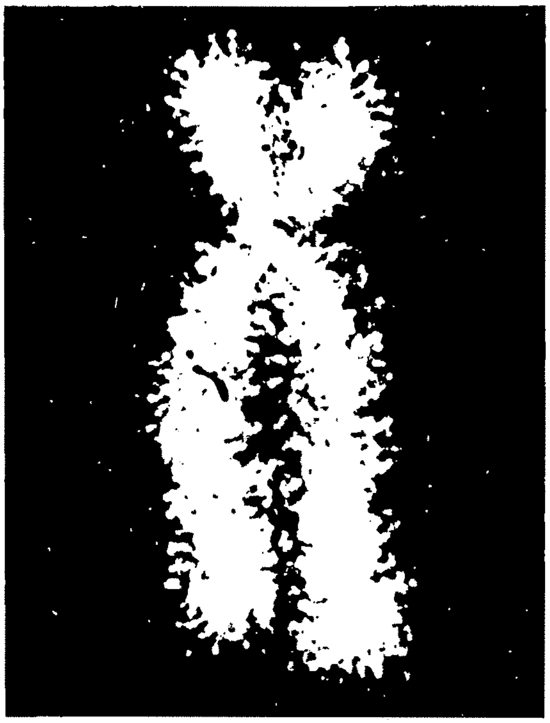

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De Vitae Origine

The Origins of Life on Earth. By L. E. Orgel. Pp. 237. (Chapman and Hall: London, June 1973.) £3 boards; £1.95 paper.

Origin and Development of Living Systems. By J. Brooks and G. Shaw. Pp. xi+412. (Academic: London and New York, May 1973.) £5.

THE arrival of the space age has rocketed the interest in the study of the origin of life to new heights. The search for life beyond the Earth, the declared goal of space biology, is intimately associated with the scientifically larger question of the origin of life in the Universe. The Oparin–Haldane hypothesis of chemical evolution has now become the focus of extensive and sophisticated investigations in many laboratories around the world.

The awareness of the general public and the interest evinced by the non-specialist in science has created a demand for the latest information on the subject in what augurs to be a flood of publications on the beginnings of life. Orgel has written, not for the professional biologist or chemist, but for the freshman in college, the advanced high school student and the general reader, who has a limited knowledge of chemistry and molecular biology. Orgel himself is one of the leading practitioners in the chemistry of the origin of life. A theoretical chemist, who has turned to biochemistry and organic chemistry, he has successfully elucidated a number of chemical pathways presumed to be necessary prerequisites for the emergence of life.

From a discussion of the historical background he gives an account of the history of the Earth's primitive atmosphere and the sources of energy necessary for the synthesis of organic compounds. He then describes the processes which gave rise to the building blocks of life. Now comes the opportune moment to examine our concept of life, before embarking on a search for life beyond the Earth.

I was disappointed that a good portion of the book has been spent on the background necessary for understanding the problem. I missed the excitement and thrill which I would have expected in picking up a book on the origin of life, written by a scientist actively engaged in its study. But the clarity of presentation, and the thoroughness of the discussion on the critical issues, make this book a valuable addition to the shelves of the general reader interested in an up-to-date and succinct account of current thinking on the problem of life's beginning.

Brooks and Shaw, on the other hand, have approached their study of the origin of life from a very different point of view. Having been involved in the study of pollen grains and their relationship to microspores, they became interested in the ancient Precambrian sediments. These studies led them to the analysis of some of the oldest sediments on Earth. According to their own admission, they felt that there was a great lack of knowledge among chemists, both in the organic and inorganic fields, about the relationship of the geochemical aspect to the problem of the origin of life. Brooks and Shaw present the geochemical background to chemical evolution in readable fashion.

The book starts with an account of the Universe and the evolution of the Earth and its atmosphere. The authors then proceed to the chemicals of life, and finally to an analysis of the Precambrian fossil record and the story of carbonaceous chondrites. Although this book is designed for the non-specialist and the general reader, a great deal of the material would be of value to the investigator and advanced student.

Of major interest are the chapters dealing with the Precambrian fossil record. Here I was delighted with the note of personal involvement. The excellent pictures provide a refreshing dimension to a stimulating discussion.

The weakness of the book is that the authors, on the one hand, have attempted to cover far too much within a limited number of pages, and, on the other, aimed at too large an audience. If the book was intended for the non-specialist, some of the chapters, though well written, may be above his grasp.

CYRIL PONNAMPERUMA

Early quanta

Quantum Mechanics: New Approaches to Selected Topics. By H. J. Lipkin. Pp. xv+465. (North Holland: Amsterdam and London; American Elsevier: New York, 1973.) Dfl. 95; $33.30.

THIS book grew from the lecture notes accrued by the author while giving post-graduate courses on quantum mechanics over a decade both in the United States and in Israel. It differs considerably from many of the standard texts intended for use with such courses. It comprises six monographs on selected topics such as the Mössbauer effect, many body quantum mechanics, Kaon physics, scattering theory, Feynman diagrams and relativistic quantum mechanics. These are presented at a much earlier level than in the conventional texts alongside which they are intended to be read.

The first is based on Dirac's use of the concept of photon polarisation as a general introduction to quantum mechanics. The idea of states of polarised light leads on to the development of a matrix representation, essentially Jones's calculus. There are other texts such as *States, Waves and Photons* by Simmons and Guttmann which are both more readable and comprehensive in their coverage of this topic. Some reference by the author to Jones and Mueller matrices might be useful as reference.

The second monograph, comprising three chapters, deals with momentum transfer with specific reference to the Mössbauer effect. Here a clear and concise listing is given which includes details of earlier experiments on resonance phenomena. Momentum transfer within a solid matrix, involving phonon interactions and nuclei, is then described with emphasis on beta decay and electron scattering by complex nuclei. This monograph is mainly concerned with the concept of a form factor and requires only an understanding of a free particle and harmonic oscillator in order to be led up to a formal treatment of second quantisation.

The treatment of many particle quantum mechanics follows in the next monograph which deals in turn with fermions and the application of fermion pairs to superconductivity. The concepts of time-dependent perturbation theory are then introduced in the next monograph on the treatment of Kaon decay. A chapter on one-dimensional scattering models provides an excellent introduction to this important part of quantum mechanics and is followed by three chapters dealing with the many body interaction problem. A comprehensive presentation of Thomas-Fermi and Hartree-Foch models is followed by a treatment of elementary excitation processes including grant resonances. The final section in this monograph introduces the reader to spectral and Green's function without entering fully into their applications.

The reader is introduced next to time-dependent perturbation theory through Feynman diagrams, using third order 'bremsstrahlung' processes as an example. The polaron is also used as an example of a quantum field approach to solid state physics.

The final monograph introduces the reader to symmetries, invariance and relativistic quantum mechanics. Starting with the Dirac equation, the reader is introduced to gauge transformations, the Lorentz group as a case of symmetry transformation and finally to charge conjugation.

Each monograph in this excellent book is introduced by a summary and ends with a problem test. The book is recommended to graduate students and research physicists looking for a pedestrian but fairly informal treatment of these topics.

D. W. GOODWIN

Chromosomes decide sex

Genetics of Sex Differentiation. By Ursula Mittwoch. Pp. xi+253. (Academic (Harcourt Brace Jovanovich): New York and London, June 1973.) $16.50.

THAT sex was another example of the transmission of hereditary factors rather than an environmentally controlled duality was probably first postulated by Mendel but was still a pertinent question at the turn of the century. In the companion book, *Sex Chromosomes* (Academic, London; 1967), Ursula Mittwoch briefly reviewed the early concepts of how sex is determined, which followed the discovery of the sex chromosomes. She dealt in detail with the mechanism in plants and animals, presenting the view that sex determination may be a process not depending on specific differences of protein production.

The present book is a general and useful introduction to the subject of sex development and its control especially in vertebrates and amplifies this concept. It is important, at this stage, to distinguish between sex determination (the primary event that decides on the type of gonad that an embryo makes) and sex differentiation (the subsequent effects of the gonad on embryogenesis of the other sex structures). The aim of the book is to give substance to the idea that sex determination results from an epigenetic control that primarily resides in whole chromosomes or chromosome segments. These, because they are visibly different, are assumed to have an effect on development "which cannot be accounted for in terms of genes". In other words the suggestion is that sex chromosomes determine sex by functioning as larger units rather than through the action of individual genes: this is the first part of the hypothesis. For its second part the author turns to non-genetic DNA, its redundancy, variation and function and to the view that sex chromosomes may control sex determination by affecting differentially the rates at which cells divide and grow during embryogenesis.

The thesis that Mittwoch presents is contrasted with other hypotheses involving discrete genic determinants, as the mainspring for gonadal differentiation, and she marshals various lines of support for it. Examples are taken from the effect of heterochromatic B chromosomes on mitotic rates in plants, the effect of X chromosomes on the mitotic cycle of human cells *in vitro*, the effect of growth rates on embryonic differentiation, particularly the analysis of transdetermination of *Drosophila* imaginal disks, and finally the effect of the various environmental influences on embryonic differentiation. Indeed, the bulk of the first six of the seven chapters of the book is an introduction to the thesis developed in the final chapter.

Irrespective of whether the hypothesis which this book discusses will ultimately turn out to be right or wrong, it is important that attention has been drawn to it. Experiments of nature should be scrutinised and experiments should be devised to see if they falsify the idea, but until then it should rank with the discrete gene hypotheses of sex determination.

PAUL E. POLANI

Insects of Europe

A Field Guide to the Insects of Britain and Northern Europe. By Michael Chinery. Pp. 352. 60 plates. (Collins: London, November 1973.) £2.95.

THIS latest addition to the Collins' Field Guide series follows their recent pattern of including Europe, in this case Europe north of the Alps. But anyone expecting to identify any insect encountered, either in this country or while holidaying on the Continent, will be sadly disappointed. This field guide only claims to enable people with no previous knowledge of the subject to first make sure that what they have found is an insect, and second to place it in the correct order and either the appropriate superfamily or family. For further identification to species a fairly extensive bibliography is provided, although some may be recognised from the superb colour plates. Those depicting lepidopterous larvae are, however, rather garish and are irritatingly interleaved with the glossary.

The glossary surprisingly omits the only obvious competitor, *The Oxford Book of Insects*. Although this new guide does not contain the comprehensive coverage of butterflies and dragonflies, it has advantages over the other in containing simplified keys, well illustrated with thumbnail sketches. The author admits that these ignore the 'odd-man-out' but they are nonetheless very valuable.

There is an excellent general introduction to entomology containing essential details of anatomy and biology, with notes on collecting and preserving insects. Unfortunately the Code for Insect Collecting is printed on the dust jacket which may become separated from the book. There is a concise chapter on classification and taxonomy and a list of entomological suppliers. It is unfortunate that the Lepidoptera section of the revised Kloet and Hincks could not be used, but the use of archaic Psocoptera nomenclature is less forgivable. Little content would have been lost by covering only British insects, although I believe there is a similar need for such a guide on the Continent. The book will sell on first impressions alone, and is extremely good value.

R. COLIN WELCH

Theory of athletics

The Mechanics of Athletics. By G. Dyson. Pp. 240. (University of London: London, September 1973.) Boards £3.30; paper £2.20.

THIS book, by the Director of Physical Education at Winchester, formerly Chief National Coach to the Amateur Athletic Association, is one that every athlete wishing to add to his skill should continually study. Mr Dyson has vast experience of athletics and coaching, and makes clear at the outset his creed that the theoretical understanding of what one is supposed to be doing is the key to improvement. "Those enamoured of practice without science [theory] are like a pilot without rudder or compass, never certain where he is going", he quotes (from Leonardo da Vinci). But he well understands that the theory must be allowed to sink in and become so much part and parcel of the method of the performer that he comes to do the right things automatically, and it is the task of a coach to induct such theory with skilful application. This of course holds for most if not all instruction: the existing level of a pupil's state of understanding and attainment must be assessed and the philosophic pill gilded accordingly.

After first lucidly expounding what is needed to be known for his purposes about motion and force, and about rotatory motion with its less obvious centripetal and gyroscopic consequences, Mr Dyson takes his readers through everything that he has found to be essential in comprehending the correct principles of running, hurdling, jumping (high and long), pole vaulting, shot putting, and throwing the hammer, discus, and javelin. No great prior knowledge of mechanics is required from the reader. Any athlete who has come to a steady state of achievement, yet nevertheless feels he ought to be able to do better, may well find the secret to his making fresh progress in the relevant chapters of this book. It has seen five previous editions, been translated into several languages including Japanese (who mean business in the sporting world, be it noted), and not surprisingly is required reading in many universities.

It is hard to know how many readers of *Nature* may also be interested in athletics, but if they are not themselves, their children or friends may be, and whether or not these have access to coaching at high level, this book could well make an admirable scientific gift of permanent value that would do much to add to their enjoyment of athletics both through greater appreciation of the fundamentals and improvement of their own performance.

R. A. LYTTLETON

matters arising

30 S DNA: intermediate or artefact?

Sir,—Goldstein and Rutman[1] have pulse-labelled the DNA of Ehrlich ascites cells growing intraperitoneally in a mouse with either ^3H or ^{14}C-thymidine. The DNA was subsequently extracted from the cells and its molecular weight measured on gradients of alkaline sucrose.

After short pulse times (<2 min), the labelled DNA had a sedimentation coefficient of 9S indicating the presence of short intermediates in DNA replication ("Okazaki" fragments[2]). After longer times (10–30 min) they observed labelled DNA which sedimented somewhat more slowly than the bulk DNA (30S as opposed to 44S) and they claimed that this represented a discrete intermediate in the replication of DNA. This interpretation is improbable.

If a long molecule is labelled at one end (as happens with a short pulse label) and then sheared, the labelled molecules will always appear to have lower molecular weights than the bulk material. This artefact is a result of the different labelling patterns of pieces that derive from molecules labelled only at the ends, as compared with molecules labelled throughout their lengths. This effect has been described qualitatively in ref. 3 and a complete mathematical analysis can be found in ref. 4.

The observation of apparently slightly smaller lengths of pulse-labelled DNA (for example, 30S as opposed to 44S) is therefore not in itself evidence for a special class of DNA molecules. It merely demonstrates that the label goes on to the ends of growing DNA strands. This criticism does not apply to the observation of 9S DNA. The most plausible interpretation of the data is that there is an intermediate in DNA replication of about 0.4×10^6 daltons and that this is linked directly on to the end of the growing DNA molecule.

Yours faithfully,

M. G. Ormerod

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Royal Cancer Hospital,
Clifton Avenue,
Belmont, Sutton, Surrey

A. R. Lehmann

MRC Cell Mutation Unit,
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Drs Goldstein and Rutman respond: The points from our study[1] of 'self-chase' Ehrlich ascites tumours which supported the DNA replication model involving sequential tandem ligations of 9S→30S and 30S→44S were: (1) maximal radioactive pulse-labelling of new 9S DNA by 2 min; (2) sharp peak transitions in alkaline sucrose gradients of labelled DNAs from alkaline-lysed cells, with no broad envelope of DNA sizes observed (2–120 min); (3) time of appearance of bulk-size labelled 44S DNA, 20–30 min, was best explained by ligation of 20 of the 9S chains to form 30S DNA and further ligation of 3 of 30S to form 44S; if 9S were directly linked to the end of a growing, long molcecule, as proposed by Lehmann and Ormerod[3,4], it should have taken 120 min to form 44S with an initial polymerisation rate of 600 deoxynucleotides per min; (4) at that rate of synthesis, excess DNA per cell would have been made in a 10 h S phase[1].

If ligation processes are under the control of replication fork(s)[1], before any ligation could begin, RNA primer molecules, to which Ehrlich cell Okazaki fragments are probably covalently linked[5,6], would have to be removed and replaced by DNA, as described for *E. coli*[7]. These latter mechanistic requirements with respect to 9S DNA taken in conjunction with our approximate kinetic analyses of 44S formation indicate that, like 9S, 30S DNA is a discrete replicative intermediate and not an artefact. The crux of this discussion rests on our choice between alternative interpretations of the data.

We have subsequently shown that bifunctional nitrogen mustard interferes with both synthesis of 9S DNA chains and their eventual step-wise conversion to 44S through 30S DNA[8].

Yours faithfully,

Norma O. Goldstein
Robert J. Rutman

Department of Animal Biology,
School of Veterinary Medicine,
University of Pennsylvania,
Philadelphia,
Pennsylvania 19174

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Impact erosion by jets of dilute polymer solutions

Sir—Results described in the letter by Kudin *et al.*[1] increase the number of types of materials which have been cut by an impacting jet containing traces of soluble polymers. The hypothesis propounded by the authors, that increase in erosion capability is due to the rigidity of large aggregates of the polymer when subjected to high loading rates is not, however, correct.

In work carried out at the Universities of Leeds and Missouri-Rolla[2,3] work on polymer addition has shown an improvement over plain water jets in impact erosion of rock (Fig. 1). The fluid was filtered at 10 μm before entering the pumping system. This would hold back any large aggregates which Kudin *et al.*, suggested act as solid particles at short time scales and cause the

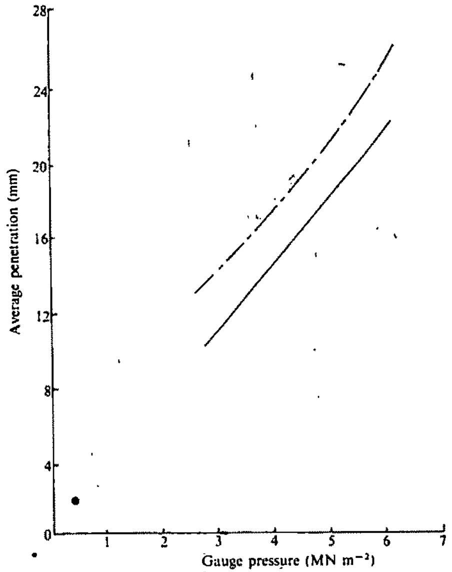

Fig. 1 Comparison between penetration of a water jet and jet containing Polyox into sandstone under varying pressure (after ref. 2). ——, Ordinary jet; – – –, with 0.1% Polyox.

erosion. The likely cause of the improvement is the increased cohesion of the jet stream, rather than any change in physical property of the fluid—such changes in density and viscosity are negligibly small in the concentrations used —or solid particle behaviour of polymer and associated solvent. That this is so is shown by the increasing effect of the polymer on jet cutting ability as the target is moved away from the nozzle (Fig. 2). Photographic evidence of the improvement in cohesion is available[4] for studies where the surrounding fluid is air and this improvement may be enhanced when the jets are submerged as apparently was the case in ref. 1. The presence of the central core on the surface of the target during the erosion process (Fig. 2c of ref. 1) is a further indication that the impacting fluid, rather than a series of solid impacts as suggested[1] is the cutting agent since such a cone is a feature of water jet erosion[5].

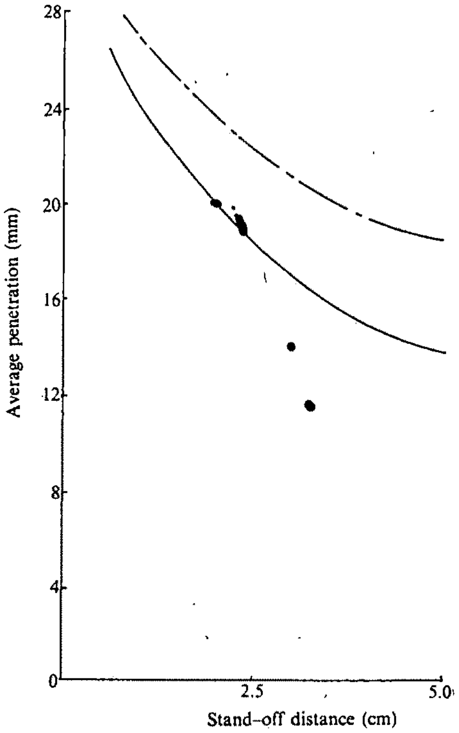

FIG. 2 Comparison between penetration of a water jet and a jet containing Polyox into sandstone with increase in target distance (after ref. 2). ——, Ordinary jet; — — —, with 0.1% Polyox.

DAVID A. SUMMERS
JACQUES L. ZAKIN

University of Missouri-Rolla
Rock Mechanics and Explosives
Research Center

Received December 14, 1973.

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The individual and the information problem

THE skewness of the distribution of journals in which individual scientists and research institutes as a whole publish their findings, shown by Blaxter and Blaxter[1] in their recent paper, will come as no surprise to most research librarians. A similar phenomenon of the distribution of journals relevant to particular specialities has been known for a number of years.

As long ago as 1927, Gross and Gross[2], studying chemical literature, developed a theory of evaluating periodicals based primarily on the assumption that the value of a periodical to a scientific worker is in direct proportion to the number of times it is cited in the scientific literature. The results obtained showed this typical distribution, with a relatively small number of periodicals containing a high percentage of the papers thought to be relevant to the subject, but with the subject's total literature widely dispersed through many journals. A similar conclusion was arrived at by Bradford[3] and later by others working on Bradford's Law[4,5]. Over the past 25 yr the literatures of many different subject fields have been analysed, and all show similar distributions[6-10]. Garfield[11] has recently shown this same skew distribution to be a property of the scientific literature as a whole, important work being concentrated in relatively few journals.

Blaxter and Blaxter's estimations for the optimum size of a working library satisfying 90% of the needs of 90% of its users could probably be reduced even more, as it is very likely that several of the twenty-five journals relevant to each of the five subject fields would be the same. The 25 yr estimated working life of the library stock only holds true if the current research interests of the workers do not change appreciably during this time. When major new fields are entered new journals are needed. Similarly, journals supporting superceded research work should be pruned from the library stock long before they are 25 yr old.

All librarians are very well aware of the costs of subscribing to, and maintaining long back-files of journals, and much bibliometric work has been carried out in an attempt to minimise these costs. This indication that users appreciate these points too makes a welcome alternative to their more usual requests for yet more new journals.

Yours faithfully,
PETER THORPE

Research Library,
The Boots Company Ltd,
Nottingham, NG2 3AA

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(319)

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(353)

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Department of Pre-Clinical Studies

1. SENIOR LECTURER OR LECTURER (Grade II) in ANATOMY OR COMPARATIVE ANATOMY
2. SENIOR LECTURER OR LECTURER (Grade II) in MICROBIOLOGY AND/OR PATHOLOGY

Applications are invited for the above full-time posts to commence as soon as possible. Candidates must have suitable academic and/or professional qualifications and some teaching experience.

Initial salary dependent upon good qualifications and experience within the Burnham Scale ranges: £3,291-£3,655 for Senior Lecturer, £2,515-£3,243 for Lecturer Grade II. The posts are pensionable under the college's own scheme.

Detailed applications, including a curriculum vitae and naming two referees, should be sent to:

The Dean,
Anglo-European College of Chiropractic,
2 Cavendish Road,
Bournemouth,
Hampshire BH1 1RA.

(262)

UNIVERSITY
OF DUBLIN
Trinity College

JUNIOR LECTURER IN APPLIED MATHEMATICS

Applications are invited for the above post. Candidates should have a research interest in one of the following areas: Applied Mathematics, Theoretical Physics, Mathematical Biology or Numerical Analysis.

Salary scale: £1,969 by £126 to £2,473.

Marriage and children's allowances are paid and there is a non-contributory F.S.S.U.-type Pension Scheme.

Further particulars and application forms may be obtained from:

The Staff Secretary,
West Theatre,
Trinity College,
Dublin 2,

who will receive completed applications up to February 18, 1974.

(177)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

CHAIR OF PHARMACOLOGY

The University of Nottingham invites applications from registered medical practitioners for a new Chair of Pharmacology which has been established initially within the Department of Physiology in the Medical School. The salary will be within the appropriate Professorial range with membership of F.S.S.U.

The Department of Physiology was established in the new Medical School in 1968 and is currently housed in recently built accommodation. Very good physical facilities will be available for the Professor of Pharmacology and his supporting staff in the new Medical Sciences Building which is approaching completion. The first medical students entered the University in October 1970 and clinical training began in 1973. It is hoped that the Professor of Pharmacology will be able to take up his appointment as soon as possible.

Full particulars of the appointment, together with copies of relevant documents concerning the Medical School and application forms, may be obtained from the Senior Assistant Registrar, Medical School, University Park, Nottingham NG7 2RD. Applications should be returned by March 1, 1974. (286)

UNIVERSITY OF OXFORD

DYSON PERRINS (ORGANIC CHEMISTRY) LABORATORY

DEPARTMENTAL ASSISTANT

Qualification: Organic chemist of postdoctoral status. Appointment: 2 years, extension to 3 possible. Duties: Collaboration with one of the research groups and in the design and supervision of undergraduate experiments. Salary: from £1,929 p.a. plus FSSU.

Starting date: October 1, 1974. Applications with 2 referees' names to Administrator, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, as soon as possible before February 25. (304)

AUSTRALIAN NATIONAL UNIVERSITY

THE JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

FELLOWSHIP IN MICROBIOLOGY

Applications are invited for a position of Fellow, tenable in the Department of Microbiology (Head of Department, Professor G. L. Ada).

Groups in the Department work in the fields of animal virology and cellular immunology. It is preferred that applicants should have had postdoctoral experience in the field of antigen structure and modification.

The salary range for a Fellow is $A10,432 to $A14,288 per annum and the appointment is for an initial period of five years; thereafter, he may be reappointed until retiring age. A Fellow is entitled to one year's study leave on full salary, plus a contribution towards travel and other expenses, in every seven years of service.

Superannuation is on the F.S.S.U. pattern with supplementary benefits. Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra.

Enquiries on academic aspects of the position should be addressed to Professor G. L. Ada in the University. Information about the method of application should be obtained from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close on **February 22, 1974.** (344)

UNIVERSITY OF PAPUA NEW GUINEA

Applications are invited for post of (a) SENIOR TUTOR or (b) TUTOR IN GEOLOGY, tenable as soon as possible. Preference will be given to applicants in the fields of historical palaeontology. Appointee must be able and willing to teach all aspects of geology at an introductory level. Salary scales: (a) A$6,715-A$7,397 per annum, (b) A$5,463-A$6,144 per annum plus dependants allowance of A$360 per annum. (These rates include an overseas allowance. £1 sterling=A$1.49.) Conditions include provision of housing, study leave, annual leave fares, F.S.S.U. superannuation.

Applications (2 copies) should include particulars of age, nationality, marital status, academic record, qualifications and experience, names and addresses of 3 referees, a recent small photograph, and an indication of estimated starting date. Further details and conditions of appointment are available from K. R. Long, Secretary, P.O. Box 4820, University, Papua New Guinea. Closing date February 14, 1974. (275)

UNIVERSITY OF OXFORD

SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

UNIVERSITY LECTURESHIP IN IMMUNOLOGY

The University proposes to appoint a Lecturer in Immunology. The Lecturer will be required to give instruction in Immunology at both introductory and advanced level and to pursue research in the subject. The salary will be according to age on the scale £1,929-£4,863. The successful candidate, if a man may also hold a fellowship at Keble College, while if a woman, a fellowship at St Hilda's College.

Further particulars may be obtained from Professor H. Harris, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE, to whom applications (five copies) with curriculum vitae and the names of two referees should be sent not later than February 28, 1974. (306)

AGRICULTURAL RESEARCH COUNCIL

SCIENTIFIC OFFICER/HIGHER SCIENTIFIC OFFICER required to join multidisciplinary research team studying new areas in the physiology and biochemistry of growth and development of meat producing animals. An interest in the metabolism of hormones and drugs would be an advantage.

MINIMUM QUALIFICATIONS: Pass degree, H.N.C. or equivalent (preferably physiology, chemistry, or related subjects).

SALARY:
Scientific Officer scale £1,318-£2,177;
Higher Scientific Officer scale £2,076-£2,667,
depending on qualifications and experience, a minimum of 5 years' post-qualifying experience being required for appointment in the H.S.O. grade. Optional superannuation scheme.

Application forms (quote Ref. BP36): Secretary, Meat Research Institute, Langford, Bristol BS18 7DY. (282)

ASSISTANT EXPERIMENTAL OFFICER required for Chemistry Department, University of Reading, as soon as possible, to work on the development of advanced teaching experiments in Physical Chemistry, the training of Junior technical staff and research in gas kinetics. Hons degree in Chemistry or GRIC desirable. Suitably qualified applicants may be permitted to work for a higher degree. Salary in the scale £844-£2,177 p.a.—Apply, quoting Ref TN8, with names of two referees and details of qualifications and experience, to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH. (321)

Ministry of Agriculture, Fisheries and Food
Fisheries Laboratory, Lowestoft

Chemist

■ In Hydrographic Section ■ Analyse trace metals in sea water ■ Sea-going duties involved ■ Opportunity to improve and develop techniques.

□ HNC/degree or equivalent in Chemistry □ Knowledge of atomic absorption spectroscopy an advantage □ Age under 27 □ Appointment as Scientific Officer (£1318–£2177) □ Ref: SB/8/AD □ Closing date 14 February 1974.

□ Application forms from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Ministry of Agriculture, Fisheries and Food
Pest Infestation Control Laboratory, Tolworth, Surrey

Chemist/Physicist

■ Join small team within Bio-Chemistry Section providing Mass Spectrometric service to this and other Ministry laboratories ■ Identify pesticide residues in tissues of wildlife casualties caused by agricultural chemicals ■ Take charge of day to day running of AEI MS.30 Double Beam Instrument ■ Scope for development work and original investigations.

□ Degree/HNC or equivalent in Chemistry or Physics □ Experience of spectroscopic techniques advantageous – particularly practical application of mass spectrometry □ Age under 27 □ Appointment as Scientific Officer (£1408–£2267) □ Ref: SB/5/AF □ Closing date 18 February 1974.

□ Application forms from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Department of Health & Social Security
British Pharmacopoeia Commission Laboratory, London.

Chemists/Pharmacists

■ 2 posts concerned with official standards ■ Modern analytical methods ■ Opportunity to develop new techniques ■ Committee work and participation in conferences and symposia.

■ Post A: Examination of proposed specifications of medicinal products ■ Analytical control of materials for use as BP Authentic Specimens.

■ Post B: Formulation and associated analytical problems ■ Liaison with industry, hospitals and universities.

□ 1st/2nd hons degree or equivalent in Chemistry or Pharmacy □ 4 years relevant postgraduate experience □ Age normally 26–31 □ Appointment as Senior Scientific Officer (£2790–£3815) □ Ref: SB/3/KD □ Closing date 18 February 1974.

□ Application forms from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

(339)

Science group
CIVIL SERVICE

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF ORTHOPAEDIC SURGERY

Applications are invited from SCIENCE GRADUATES for the post of Research Assistant to undertake an investigation of the factors stimulating or retarding the growth of rodent long bones. Experience of autoradiography would be an advantage. The project is supported by the United Liverpool Hospitals Medical Research Committee and it is anticipated that it will continue for three years.

The salary will be in the range £1,572-£1,830 per annum. However, if the appointed person registers for a higher degree the salary will be in accordance with the scales currently employed by the S.R.C.

Applications, together with the names of three referees, should be received not later than January 31, 1974, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref. RV/N/80652. (258)

UNIVERSITY COLLEGE OF WALES

ABERYSTWYTH

DEPARTMENT OF AGRICULTURE

Applications are invited for two posts of RESEARCH ASSISTANT. Salary: £1,209 per annum.

(1) To join a team which has an extensive research programme in early potatoes.

(2) To assist in the conduct of experimentation at the Department's Trefloyne Field Station, near Tenby, Pembrokeshire.

Applicants should have a good honours degree in Agriculture, Crop Production, Agricultural Botany or Plant Sciences. Registration for a higher degree is possible.

Application forms and further particulars may be obtained from the Registrar, The University College of Wales, Old College, King Street, Aberystwyth, to whom they should be returned by February 15, 1974. (332)

TECHNICIANS

International Serum Laboratories, part of the Hoechst (UK) Pharmaceutical Division, have three immediate vacancies for their site at Mill Hill, N. London.

The first post involves sterility and analytical testing. Applicants, male or female, should have 'A' levels or ONC.

The second involves vaccine blending including sterilisation and operation of bulk process equipment.

The third involves the operation of fermentation and antigen processing equipment.

Please contact:
Mrs M. C. Hannay,
Personnel Officer,
HOECHST UK LIMITED,
Salisbury Road, Hounslow West, Middlesex.
Tel: 01-570 7712 Ext 3082

Applicants for these latter posts should be A.I.M.L.T., or equivalent. Experience of similar work is useful but not essential.

We offer good salaries plus first class benefits which include an annual bonus, contributory pension scheme and free health scheme.

(335)

unilever research

Biologist

We are looking for a Biologist to join a team of Biophysicists, Physicists, Physical Chemists and Biologists working on the effect of materials such as detergents on the structure and formation of skin.

The exact field of his/her primary degree is less important than a good knowledge of cell physiology and general biochemistry. Experience of work in skin or related systems or on cell adhesion would be relevant to our needs.

The candidate should be prepared to learn and use physical and chemical techniques to investigate, for example, the effect of detergents on cell adhesion but should remain a Biologist and be able to interpret results in biological terms.

The successful candidate will preferably be aged under 30, have a Ph.D. and at least one year's additional experience.

The Company operates a progressive salary and promotion policy, good superannuation and life cover benefits and, where appropriate, will assist with removal expenses.

The Laboratory is situated on the Wirral peninsula, a few miles from the sea and convenient for Chester, Liverpool and North Wales.

Write for an application form, quoting ref: PS. 432, to:

Recruitment & Placement,
Unilever Research,
Port Sunlight Laboratory,
Port Sunlight,
Cheshire L62 4XN.

(333)

BAKER MEDICAL RESEARCH INSTITUTE

MELBOURNE, AUSTRALIA

(RE-ADVERTISEMENT)

The Trustees of the Thomas Baker, Alice Baker and Eleanor Shaw Medical Research Institute invite applications for the position of:

DIRECTOR OF THE INSTITUTE

The present Director will be retiring from appointment in December 1974.

THE INSTITUTE

The Baker Institute is an autonomous organisation situated in the grounds of Alfred Hospital and adjacent to the Clinical Sciences Building of Monash University Medical School. The Institute is affiliated with Monash University which means that the research facilities of the Institute are available for promoting the teaching of both undergraduate and postgraduate students of the University, up to Ph.D. level. For both bodies it makes possible the integration of trained personnel and the research facilities of both the University and the Institute, for the benefit of medical education and research. The main financial support of the Institute is presently derived from the Thomas Baker (Kodak), Alice Baker and Eleanor Shaw Benefactions.

The Institute building has recently been rebuilt, all laboratories are air-conditioned and equipped for work in biochemistry, physiology, pharmacology and histology. The laboratories of Alfred Hospital Clinical Research Unit are presently housed in the Institute building. The building is linked with the new Alfred Hospital Ward Block now under construction. Arrangements with the Hospital will permit the successful applicant access to clinical facilities, including beds, if such are appropriate to the research programme.

REQUIREMENTS

Applicants must be medical or biological scientists with research experience, demonstrated ability in team leadership and some appropriate administrative experience.

CONDITIONS

- The appointment is for at least five years and will be reviewed not later than four years after the appointment, when it may be extended as appropriate.
- A generous salary is contemplated, and will be based on qualifications and experience.
- Superannuation is available under Institute Scheme.
- Commencing date January 1975 or later by negotiation.
- Assistance will be given in meeting the cost of removal expenses to a successful applicant not resident in Melbourne and in some circumstances with the cost of repatriation.

Further details may be obtained from the Director, Dr. T. E. Lowe.

Applications close with the undersigned on April 30, 1974.

G. B. CANHAM,
Secretary.

Baker Medical Research Institute,
Commercial Road,
Prahran, Victoria 3181,
Australia. (193)

UNIVERSITY OF SURREY

DEPARTMENT OF METALLURGY AND MATERIALS TECHNOLOGY

Applications are invited for the posts of Redland Research Fellow and Research Officer tenable in the Department of Metallurgy and Materials Technology at the University of Surrey.

Applicants for the first of these posts should have a Ph.D. or equivalent experience in Materials Technology, preferably in composite materials, ceramics or plastics. The Fellow appointed will undertake research on materials of interest in construction and building and in detail will be concerned with the property/structure relationship of structural materials. He will be expected to liaise closely with Redland Ltd.

Applicants for the post of Research Officer should have a degree or equivalent training in some aspect of materials science or technology and an interest in or experience of fibre composite materials. Experience in the effect of fabrication routes on the properties of fibre reinforced composites would be an advantage.

Remuneration would depend on age and experience and for the Research Fellow would be made in relation to the University Lecturer scales, in the range £1,929 to £2,718, and for the Research Officer an appointment would be made within the salary range £1,900 to £2,400.

Applications should include a curriculum vitae and be sent to Professor J. E. Bailey, Department of Metallurgy and Materials Technology, University of Surrey, Guildford, Surrey GU2 5XH. (287)

ROYAL POSTGRADUATE MEDICAL SCHOOL. SENIOR TECHNICIAN I required in Department of Surgery to work in the field of cell-mediated immunity; previous relevant experience essential. Qualifications: H.N.C. or equivalent. Starting salary up to £1,956 per annum.—Applications to the Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, DuCane Road, London W12 0HS, quoting Ref. 3/298/N. (268)

Nottingham Area
Cytogenetics Service

Non-Medical Principal Scientific Officer

Applications are invited from cytogeneticists for the above appointment. The individual appointed will be responsible for a diagnostic chromosomal service which operates in conjunction with the newly-created Genetic Counselling Service in Nottingham.

The service will operate from the City Hospital, Nottingham, and laboratory accommodation is associated with the recently established University Departments of Obstetrics and Gynaecology, and of Child Health. There will be opportunities for association with the University departments. Applicants should have a higher degree and experience in a cytogenetics service unit. Salary in the range £3,516 to £4,599.

Application forms and further details can be obtained from the Secretary, Nottingham and District Hospital Management Committee, Sherwood Hospital, Hucknall Road, Nottingham NG5 1PD.

(310)

1666 TRUMAN BREWERY

Biochemist

We require a Graduate to join our small R & D team to assist in the formulation of new products and the development of improved brewing techniques.

Applicants should have had at least 2 years industrial experience in fields involving organic chemistry and a knowledge of enzymes would also be useful.

We are now part of a very large brewing group and there are excellent opportunities for development and promotion.

We can offer good fringe benefits and a starting salary according to qualifications and experience of not less than £2,500 per annum.

Applications in writing giving brief details of qualifications and experience to:—

The Employment Officer,
Truman Limited,
91, Brick Lane, London, E1 6QN. (338)

UNIVERSITY OF MANCHESTER
RESEARCH FELLOW

DEPARTMENTS OF CHILD HEALTH AND COMMUNITY MEDICINE

Applications invited from science graduates with research experience involving the use of statistics, to participate in an epidemiological study of the 2,000 or so cases of children's cancer included in the Manchester Children's Tumour Registry. Three-year appointment funded by the National Institutes of Health, USA. Suitable training post for a career in cancer epidemiology. Starting salary not less than £1,929 p.a.

Further details and application forms (returnable by February 15) from the Registrar, The University, Manchester M13 9PL. Quote ref No 6/74/Na. (329)

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF GEOLOGY

Applications are invited from candidates with a good Honours degree and suitable postgraduate experience for the post of LECTURER IN SEDIMENTOLOGY which is tenable from October 1, 1974. Salary will be at an appropriate point on the scale £1,929-£4,548 according to age, qualifications and experience. Membership of F.S.S.U. required.

Further particulars may be obtained from the Registrar, The University, Newcastle upon Tyne NE1 7RU, with whom applications (3 copies), together with the names and addresses of 3 referees, should be lodged not later than February 23, 1974. Please quote Ref. Na. (284)

Wellcome

Information Scientist

A vacancy has arisen in the Research Information Centre for a graduate biochemist, pharmacologist or pharmacist. The Centre comprises a team of young graduates and associated staff developing information handling techniques in support of the Company's research and development effort. The Company possesses a 1903S computer and automation of information storage and retrieval is well advanced.

The successful applicant will be expected to undertake certain routine duties such as literature searching and indexing of company reports relating to research and development in the biochemical/pharmacological field. There will also be participation in project and study groups and the position requires initiative and an ability to communicate with scientific staff.

Candidates should be between 22 and 35 years of age and possess an Honours degree in an appropriate subject. Preference will be given to applicants with relevant experience and/or post-graduate qualifications.

The laboratories are situated in pleasant parkland surroundings and conditions of employment are attractive.

Applications, giving brief details of background and experience, should be sent, quoting reference U.434, to:

The Personnel Manager,
Wellcome Research Laboratories,
Langley Court,
Beckenham,
Kent BR3 3BS

1971

(352)

Microbiologist

John Wyeth & Brother Limited, an associated Company of Wyeth International of Philadelphia, U.S.A., produce a wide range of pharmaceutical and infant food products at their modern factory in Havant, Hampshire.

A vacancy exists for a Microbiologist, aged 25/45 with a degree or equivalent qualifications in Microbiology, who will be required to join a small team involved in the Microbiological Quality Control of pharmaceutical and nutritional products.

A competitive salary will be paid depending upon age and experience. The company offers a non-contributory pension and life assurance scheme. Assistance with relocation expenses will be considered.

Please apply to:
Cameron Wolstenholme,
Personnel Controller,
John Wyeth & Brother Limited,
Huntercombe Lane South, Taplow,
Maidenhead, Berks.

(315)

Wyeth

AGRICULTURAL RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE

SCIENTIFIC OFFICER required in the Microbiology Division to assist with experimental work on the physiology of bacterial spores. Minimum qualifications H.N.C. in appropriate subject. Previous training or experience in microbiology is essential and should preferably include some physiology or biochemistry of micro-organisms. Candidates should normally be under age 27. Salary scale £1,318 to £2,177 plus a supplement of 5½ per cent to offset superannuation contributions. Starting salary according to qualifications and experience. Optional contributory superannuation scheme.

Application forms and further details from the Secretary, Food Research Institute, Colney Lane, Norwich NOR 70F, quoting Reference 73/27.

(280)

WESTFIELD COLLEGE
(UNIVERSITY OF LONDON)

Applications are invited for a RESEARCH ASSOCIATE to work with Dr. J. A. Riegel on malpighian tubule function. Applicants should have completed recently, or be near to completing, Ph.D. requirements. Experience of working with isolated tissues and knowledge of electron microscopical techniques would be an advantage. The maximum tenure of the post will be two years commencing October 1, 1974. Salary will depend on experience, but will not exceed £2,385 per annum including London Weighting.

Further particulars may be obtained from the Personnel Officer (N), Westfield College, Kidderpore Avenue, Hampstead, London NW3 7ST, to whom a curriculum vitae (with the names of two referees) should be sent by February 22, 1974. (266)

BIOLOGICAL OCEANOGRAPHER MARINE BIOLOGIST

Brookhaven National Laboratory, located on Long Island about 65 miles east of New York City, seeks marine biologist with Ph.D. or equivalent and outstanding record of research to initiate a program in biological oceanography in the north-eastern coastal zone. Successful candidate will be expected to play a major role in making further appointments in this area.

Send curriculum vitae, list of publications, reprints of two recent papers, and three professional references to: Dr John M. Olsen, Search Committee Chairman, Biology Department,

Brookhaven National Laboratory,
Associated Universities Inc.,
Upton, New York 11973

An Equal Opportunity Employer M/F

(X325)

PORTSMOUTH POLYTECHNIC
SCHOOL OF PHARMACY

LECTURER II IN PHARMACOLOGY

(READVERTISEMENT)

Applications are invited from pharmacists or pharmacologists to participate, mainly in the teaching of pharmacology to students of the pharmacy degrees and the Honours degree in pharmacology, and to assist with the teaching of other courses in the School of Pharmacy. A higher degree together with teaching or research experience is desirable. Excellent research facilities are available.

Salary scale: £2,515 to £3,243 per annum.

Application forms and further particulars may be obtained from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Alexandra Road, Portsmouth PO1 2QQ, to whom completed applications should be returned by February 4, 1974. Please quote Reference R50. (249)

AGRICULTURAL RESEARCH COUNCIL

UNIT OF REPRODUCTIVE PHYSIOLOGY AND BIOCHEMISTRY

Applications are invited for the post of Assistant in the Electron Microscopy section.

Candidates should have experience in EM techniques as used in biology including ultramicrotomy and routine maintenance of electron microscope. Appointment will be in the grade of Scientific Officer or Higher Scientific Officer according to age, qualifications and experience. Minimum qualifications: Pass Degree or HNC in an appropriate subject. Salary: SO £1,318-£2,177; HSO £2,076-£2,667, at least 5 years post-qualification experience is required for entry in the higher grade. Membership of contributory superannuation scheme attracts a non-pensionable allowance.

Applications, with names of two referees, to the Secretary, Animal Research Station, 307 Huntingdon Road, Cambridge CB3 0JQ. (291)

UNIVERSITY OF WESTERN ONTARIO

Applications are invited for the position of ASSISTANT or ASSOCIATE PROFESSOR. Responsibilities involve teaching undergraduate and gaduate students and conducting a research programme in biochemistry. Ph.D. and research experience in fields of physical biochemistry, membrane biology, or related areas will be given preference.

Send curriculum vitae and three references to Dr. W. C. McMurray, Secretary, Selection Committee, Department of Biochemistry, University of Western Ontario, London, Ontario, Canada N6A 3K7.

(261)

INSTITUTE OF CANCER RESEARCH: PHYSICS DIVISION. PHYSICIST required at Sutton, Surrey (with possibility of subsequent transfer to Guildford) to lead an MRC sponsored project involving the establishment and application of ultrasonic dosimetric and calibration facilities, Applicants should have a good honours degree and postgraduate experience related to ultrasonics or Medical Physics. Salary on MRC scales Grade II depending on qualifications and experience.—Apply in duplicate, giving curriculum vitae and quoting two referees, to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting Ref. No. 300/G/67. (288)

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF PHYSIOLOGY

Applications are invited for two posts:

LECTURESHIP OR SENIOR LECTURESHIP

available immediately or not later than October 1.

LECTURESHIP

from October 1 or a date in September to be agreed.

A medical qualification and/or a research interest in the central nervous system would be an advantage for either or both of these appointments.

Salary range: £1,929-£4,548/£4,368-£5,496.

Applications, together with the names and addresses of two referees, should be forwarded to The Registrar, University College, Cardiff CF1 1XL, from whom further particulars will be available. Please quote reference 0980. Closing date February 22, 1974. (318)

UNIVERSITY OF NAIROBI KENYA

Applications ar invited for the following posts in the Faculty of Veterinary Science:

1. SENIOR LECTURER IN DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY. Applicants should have a good degree in Veterinary Medicine and preferably a PhD or equivalent within the field covered by the Department. They should have teaching and research experience at university level. Preference will be given to those with specialisation and practical experience in Food Hygiene. Duties of appointee will involve the teaching of Public Health, particularly all aspects of meat, milk and water hygiene to veterinary students on the undergraduate and postgraduate level. He will also have to do research in his special field of interest and assist in the building up of the Department, its teaching and research programmes. This post will be tenable for a period of two years initially, effective from September 1974.

2. LECTURER IN DEPARTMENT OF VETERINARY ANATOMY AND HISTOLOGY. Applicants should be graduates with a higher degree and teaching experience in Veterinary Anatomy and Histology. Specialisation and research interests in one of these areas would be an advantage. Appointee would be expected to specialise in Applied Anatomy (surgical or medical) and will be given the opportunity of participating in research.

Salary scales: Senior Lecturer, K£2,256-K£3,036 p.a. Lecturer K£1,500-K£2,580 p.a. (K£1=£1.24 sterling). The British Government may supplement salaries in range £450-£950 p.a. (sterling) for married appointees or £150-£650 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. FSSU. Family passages; various allowances.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail, not later than February 21, 1974, to the Registrar, University of Nairobi, PO Box 30197, Nairobi, Kenya. Applicants resident in UK should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars of these appointments are available from either address. (294)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

FACULTY OF MEDICINE

Director of Multidiscipline Teaching Laboratories and Faculty Member

A vacancy exists for the above position to be filled, effective September 1, 1974. This post is suited to basic medical scientists with experience and interests in medical education, in particular, laboratory work including projects and electives in the cell sciences, physiology, and pharmacology. The Director will be responsible for the administration of the Laboratories and will actively participate in the planning and execution of the Laboratory activities and in other aspects of the curriculum. The Multidiscipline Laboratories have recently been built as part of the new Health Sciences Complex. There is an associated Director's research laboratory and administrative offices; the technical and support staff is of high calibre.

Rank and salary will be according to qualifications. Send application and curriculum vitae as soon as possible and, in any case, not later than February 28, 1974, to Dr K. B. Roberts, Associate Dean, Faculty of Medicine, Memorial University of Newfoundland, St John's, Newfoundland. (296)

Manager-Designate

Sterile filling and services department

Evans Biologicals Ltd. is part of the multi-million international Glaxo group.

The Sterile Filling and Services Department with a total staff of about 40, is responsible for the aseptic filling of a wide range of biological products, including vaccines, and for the provision of general services to the company's other production and technical departments. Reporting to the Technical Director, the Manager-Designate will take full control of this department following an agreed introductory period.

Candidates must be specialists in the highly technical field. They will either have gained experience, over a period of at least 5 years, built on theories and principles and have an M.Sc., F.R.I.C., M.Pharm. or the equivalent, or have longer experience based on a broad and deep practical experience and have M.P.S., B.Pharm., B.Sc., A.R.I.C., or the equivalent.

In addition to an attractive salary, which will be dependent on experience, the company offers excellent conditions of employment, including participation in the Glaxo group profit-sharing bonus scheme. Assistance with relocation expenses will be given, where appropriate.

Please write with brief career details, quoting reference U.41, to:

The Personnel Manager,
Evans Biologicals Limited,
Speke,
Liverpool L24 9JD

(334)

CENTRAL PUBLIC HEALTH LABORATORY

We have a part-time vacancy in our Salmonella and Shigella Reference Laboratory for an Honours Science Graduate, preferably in microbiology, to work in the E. Coli-Shigella Section. Hours by arrangement.

Applications to the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. (250)

UNIVERSITY OF OXFORD

DYSON PERRINS LABORATORY (ORGANIC CHEMISTRY)

POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a post-doctoral research assistantship to work on the chemistry of natural products with special reference to bitter principles. Previous experience in natural product chemistry is highly desirable.

The appointment is for up to two years with a possible extension for one further year, commencing as soon as possible. Salary dependent upon age and experience in the range £1,821-£2,223 per annum plus FSSU.

Applications, with the names and addresses of two referees, should be sent to Dr T. G. Halsall, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY. (305)

UNIVERSITY OF SHEFFIELD

TECHNICIAN (Grade 4) required for the Department of Biochemistry, to assist in biochemical research. An interest in Kinetics would be an advantage. Salary scale £1,848×63(5)—£2,163 p.a.

The working conditions and surroundings are pleasant; 37½-hour week, with excellent holidays and other conditions of service.

Please write to the Deputy Director of Services (Ref. S13), The University, Sheffield S10 2TN. (299)

The University of Leeds

DEPARTMENT OF PLANT SCIENCES

Applications are invited for the post of Experimental Officer at the Leeds University Field Station, Nr Tadcaster. Candidates should have a degree in Agriculture or Agricultural Science, and with interest in crops. The appointment will be for one year in the first instance, renewable for a further two years, with a possibility of a further extension. Salary will be within the scale £1,563-£2,187.

Forms of application and further particulars from the Registrar, The University, Leeds LS2 9JT (please quote 41A/9/D). Closing date February 6, 1974. (320)

ilea INNER LONDON EDUCATION AUTHORITY

Scientists & Engineers

Would you like to teach?

If you have a degree in pure or applied science, engineering or technology or allied subjects, or hold any other qualifications giving you qualified teacher status (such as a Diploma in Technology, or the associateship or graduateship by examination of various scientific institutions) then you are eligible to be considered for employment as a teacher of science with this Authority.

We intend to run an induction course for mature graduates and others with qualified teacher status, beginning in September 1974 and lasting until July 1975. The course will be held at Furzedown College of Education, in Streatham, and will include a basic preparation in teaching skills and in modern developments of science education. The course will include a whole term of teaching practice in an ILEA secondary school.

Those who successfully complete this course will be awarded the Graduate Certificate in Education – that is, they will be recognised as fully trained teachers.

Applicants admitted to the course will be given appointments to our temporary teaching staff from September 1974 so that they may be paid the appropriate rate for a teacher whilst they are taking the course. On successfully completing the course they will be given permanent appointments to our service and will be expected to remain in service for at least one further year.

The current Burnham salary scales provide that a good honours graduate would start at a salary not less than £1736 in London. In assessing the starting salaries, however, we should also take into account previous teaching experience, if any, and previous industrial or commercial experience, so that for mature people the starting salary could be substantially above this figure.

If you are interested in taking such a course please write to the Education Officer EO/TS.3/S, County Hall, London, SE1 7PB (including a self addressed envelope) as soon as possible, and in any case not later than 22 February, 1974.

(260)

NJALA UNIVERSITY COLLEGE
UNIVERSITY OF SIERRA LEONE

Applications are invited for LECTURESHIP IN CHEMISTRY. Applicants must possess a good first degree in chemistry and must have had some teaching experience in a university or similar institution. Preference will be given to applicants with a higher degree. Appointee will be expected to teach any branch of chemistry to general degree level. Salary scale: Le2,400-Le4,740 per annum (Le2=£1 sterling). The British Expatriates Supplementation Scheme is unlikely to be applied to these appointments. Superannuation scheme or gratuity; various allowances; family passages; biennial overseas leave.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail not later than February 11, 1974, to the Secretary, University of Sierra Leone, Private Mail Bag, Freetown, Sierra Leone. Applicants resident in U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (276)

UNIVERSITY OF HONG KONG
CHAIR OF GEOGRAPHY

Applications are invited for the above-mentioned Chair which will fall vacant in July 1974.

Annual salary (superannuable) will be within the professorial range and not less than HK$97,800. At the time of issue the rate of exchange is £1=HK$11.55 approximately.

Further particulars and application forms may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, or the Secretary to the Council, University of Hong Kong.

Closing date for applications is February 15, 1974. (278)

UNIVERSITY OF SYDNEY
LECTURESHIP IN AGRICULTURAL CHEMISTRY

The University will be especially interested in applicants with experience in the teaching of Agricultural Chemistry. Preference will be given to candidates with training in physical chemistry, especially in those aspects relevant to Agricultural Chemistry. The appointee will be required to play a substantial part in the organisation and teaching of practical classes particularly in Agricultural Chemistry I. The appointment will be available from June 30, 1974.

The position advertised is a permanent one, but it may be filled for three years in the first instance, with possibility thereafter of permanency or in certain cases return fares.

Salary range: $A8,698-$A11,982 p.a.

Applications, including curriculum vitae, list of publications and names of three referees, by February 18, 1974, to the Registrar, University of Sydney, Sydney, NSW 2006, Australia, from whom further information available. Information also available from Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF. (295)

CHEMISTRY DEPARTMENT
UNIVERSITY COLLEGE LONDON

Applications are invited for a POSTDOCTORAL RESEARCH ASSISTANTSHIP, available immediately, to work on Resonance Raman Spectroscopy. This post is financed by a S.R.C. Research Grant and is for up to two years. Salary is £1,929 plus £162 London Allowance, plus F.S.S.U. Previous experience in vibrational spectroscopy would be desirable.

Applications, naming two referees, should be sent to Dr. R. J. H. Clark, Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ. (246)

UNIVERSITY OF NAIROBI
KENYA

Applications are invited for the following posts in the Faculty of Agriculture:

1. SENIOR LECTURER IN DEPARTMENT OF CROP PRODUCTION. Applicants should have a good degree in Agriculture and a postgraduate degree in Crop Science or Horticulture. They should have teaching, research and field experience. Appointee will be expected to teach undergraduate courses in Crop Production and/or Horticulture, to supervise postgraduate students and to assist in developing the Faculty's Experimental Field Station.

2. LECTURER IN DEPARTMENT OF CROP PRODUCTION. Applicants should have a good degree in Agriculture, Horticulture or Botany with a postgraduate degree in General Agronomy, Horticulture or Plant Physiology. Experience in teaching and/or research will constitute a recommendation. Appointee will be required to undertake teaching of the production of crops to undergraduate students and to guide and teach postgraduate students.

3. LECTURER IN DEPARTMENT OF AGRICULTURAL ECONOMICS. Applicants should have a Ph.D. in Agricultural Economics, preferably with research background in tropical agriculture. Duties of appointee will involve lectures and practicals with undergraduate students and research supervision of postgraduate students. Applicants should have their main training and interest in the following fields: Agricultural Economics and Farm Management or Rural Sociology and Agricultural Extension.

Salary scales: Senior Lecturer K£2,256 to K£3,036 per annum, Lecturer K£1,500 to K£2,580 per annum (K£1=£1.24 sterling). The British Government may supplement salaries in range £450 to £950 per annum (sterling) for married appointees or £150 to £650 per annum (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. F.S.S.U. Family passages; various allowances.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be forwarded by air mail, not later than February 22, 1974, to the Registrar, University of Nairobi, P.O. Box 30197, Nairobi, Kenya. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars of these appointments are obtainable from either address. (341)

UNIVERSITY OF WESTERN AUSTRALIA
PERTH

Applications are invited for appointment to a

CHAIR IN OPHTHALMOLOGY

This new Chair has been established with the support of the Royal Perth Hospital and the Lions Save-Sight Foundation in the Department of Surgery (Professor B. N. Catchpole). Applicants should be medical graduates with a higher qualification in ophthalmology and eligible to register as medical practitioners in Western Australia. The appointee will work at the Royal Perth Hospital, which is the major teaching hospital in the University, comprising 1,004 beds. The salary for a Professor is $A19,102 per annum plus a differential of $A3,000 per annum for clinical responsibilities. Benefits include superannuation similar to F.S.S.U., fares to Perth for appointee and dependent family, removal allowance, study leave and long service leave, and housing loan scheme. Further information may be obtained from the Staffing Officer in the University or from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications, in duplicate, stating full personal particulars, qualifications, experience, and the names and addresses of three referees, should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia 6009, by February 16, 1974. (267)

BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE
ADMINISTRATIVE ASSISTANT

Applications are invited from candidates interested in the advancement of science. The work involves administration arising from the Annual Meeting, the organisation of committees and the preparation of reports. The starting salary will be related to age, qualifications and experience but will be within the UGC scale for assistant lecturers and lecturers.

Letters should be addressed to The Secretary, British Association, 23 Savile Row, London W1X 2AA, from whom further particulars can be obtained. (297)

ROYAL POSTGRADUATE MEDICAL SCHOOL. TECHNICIAN/SENIOR TECHNICIAN required in the Department of Medicine to undertake experimental studies in the physiology of carbohydrate absorption using both in vivo and in vitro techniques. Qualifications: H.N.C. degree in biochemistry/physiology or equivalent. Salary in range £1,578 to £2,304 per annum, according to qualifications and experience.—Applications to the Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, DuCane Road, London W12 0HS, quoting Reference No. 2/218/N. (272)

ORGANIC CHEMISTS

Vacancies for all levels of qualification for synthetic organic preparations. 37-hour week. 4 weeks' holiday per year. Pension scheme. Write, giving full details of past experience: Maybridge Chemical Company, Tintagel, Cornwall. (311)

RE-ADVERTISEMENT

UNIVERSITY OF OXFORD

TUMOUR IMMUNOLOGY

A post is available immediately for research on anti-tumour immunity assayed *in vivo.* It is supported by an MRC Project Grant for 1 year. The work will be shared between the Nuclear Physics Laboratory and Dr I. C. M. MacLennan's laboratory in the Nuffield Department of Medicine. It has potential practical importance in the radiotherapy and immunotherapy of cancer.

The project needs someone who has a PhD or equivalent experience with mice or cell cultures, and an interest in tumour immunology, radiobiology or cell kinetics is desirable. Candidates must not be over 30, salary up to £2,058 p.a. with FSSU.

Applications, including age, qualifications, the names of 2 referees, and a brief curriculum vitae, should reach Dr T. R. Munro, Department of Radiation Physics, The Churchill Hospital, Oxford OX3 7LJ, from whom further particulars may be obtained, by February 8, 1974. Telephone Oxford 64841 Ext. 665. (298)

THE CHINESE UNIVERSITY OF HONG KONG

Applications are invited for CHAIR-PROFESSORSHIPS IN CHEMISTRY AND MATHEMATICS.

Applicants should possess outstanding academic qualifications, have considerable university teaching and relevant research experience as well as published scholarly works of originality and merit. The University expects to fill these Chairs with candidates of high standing who have proven contributions in the academic discipline(s) concerned. Appointees are expected to give leadership in the teaching and research programmes in his field, and will be required to work also at research centres, institutes and postgraduate schools of the University. All appointees will be assigned to units directly under the University, including the three constituent Colleges of the University, namely, Chung Chi College, New Asia College and United College. Candidates who do not possess the required qualifications for appointment to Professorial level may be considered for appointment as Readers (salary scale £6,108 to £8,118 per annum). The academic year starts on August 1 and instruction begins in early September. Salary will commence on assumption of duty.

Salary: £8,308 per annum or above, depending on qualifications and experience. Long leave; superannuation benefits (University 15 per cent, appointee 5 per cent); medical benefits; housing accommodation; family passages; overseas leave.

Further information and application forms may be obtained from the Personnel Section, University Secretariat, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong. The application forms (3 copies), together with one set of the applicant's publications, and copies of recent testimonials, marked "CHAIR APPLICATION" on cover, should be returned to the Personnel Section by February 15, 1974. (343)

FOURAH BAY COLLEGE

UNIVERSITY OF SIERRA LEONE

Applications are invited for the post of SENIOR LECTURER/LECTURER IN PHYSICS. One or two posts may be filled. Research interests in geophysics or theoretical physics would be considered an advantage. Salary scales: Senior Lecturer Le4,550-Le5,400 per annum, Lecturer Le2,400-Le4,740 per annum (£1 sterling=Le2). The British Expatriates Supplementation Scheme is unlikely to be applied to this appointment. F.S.S.U. Family passages; various allowances; regular overseas leave.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail not later than February 11, 1974, to the Secretary, University of Sierra Leone, Private Mail Bag, Freetown, Sierra Leone. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P ODT. Further particulars of this appointment may be obtained from either address. (277)

APPLICATIONS ARE INVITED FOR AN appointment involving undergraduate instruction in **mineralogy** and **petrography** and interaction with the graduate programme. The Department's research activity emphasizes environmental geology and preference will be given to applicants concerned with the mineralogy and geochemistry of sedimentary environments. Rank open; salary commensurate with experience. A temporary one-year appointment is also available in **economic geology.**—Send résumé to: Dr R. N. Farvolden, Chairman, Department of Earth Sciences, University of Waterloo, Waterloo, Ontario, N2L 3G1. (210)

TECHNICAL SALES REPRESENTATIVES MICROSCOPES

Wild Heering (UK) Limited, following their decision to directly market Wild Microscope Equipment in Scotland, require the services of suitably qualified and experienced Sales Representatives.

The Representatives appointed will be directly responsible to and receive the full backing of the Wild UK Chatham Headquarters, for the continued development and expansion of the Scottish market. Preference will be given to those already working in this field and living in the close proximity to either Glasgow or Edinburgh.

Gentlemen having a background in the applications in Microscopy and previous technical selling experience with Microscopes or Allied Instruments are invited to apply in confidence.

Please write or telephone:

Mr. Barton Hollands
Manager Microscope Division

WILD
HEERBRUGG

Revenge Road, Lordswood, Chatham, Kent ME5 8TE
Telephone: Medway (0634) 64471/5

(313)

THE WELSH NATIONAL SCHOOL OF MEDICINE

(University of Wales)

Applications are invited for the post of **TECHNICIAN** in the Department of Haematology. Duties will include the estimation of serum iron and vitamin B12 levels, together with the development and evaluation of new techniques in this field.

The laboratories offer a wide range of facilities, including a laboratory computer, and provide a comprehensive service for the investigation of disorders of the blood.

Salary on the scale of £1,440–£2,292 according to age, qualifications and experience.

Application forms available from the Registrar, The Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN to whom they should be returned within two weeks of this advertisement.

(317)

NORTH EAST METROPOLITAN REGIONAL HOSPITAL BOARD

in co-operation with the University of Surrey.

The Board offers two special training posts as

BASIC GRADE BIOCHEMISTS

with the opportunity to study for an M.Sc. in Clinical Biochemistry.

Posts are available from September/October 1974 for a two-year sandwich M.Sc. course at the University. Applicants should hold or expect to obtain in 1974 a good Honours degree (or its equivalent) in Biochemistry, Chemistry or other suitable subject.

During the parts of the course spent away from the University students would work in a specified hospital laboratory in the area of the Board. Salary would be at the appropriate point on the scale commencing at £1,680 per annum and rising with annual increments to £2,112 per annum, plus London Weighting of £126 per annum where applicable. Fees whilst attending University have to be met by the students. The Certificate of Proficiency may be issued at the end of the two-year course if appropriate, i.e., after obtaining the M.Sc.

Further details and application form are obtainable from the Academic Registrar, University of Surrey, Guildford, Surrey GU2 5XH. Applications must give past examination grades. Closing date for applications March 4, 1974. (289)

UNIVERSITY OF SYDNEY

ENGINEER—FLEURS FIELD STATION

A first degree in Electrical Engineering with wide experience of solid state devices and design of electronic equipment are essential qualifications. It is very desirable that an applicant should have a higher degree in electrical engineering and should have had experience in radioastronomical work.

The successful applicant will have the responsibility for the maintenance of the electronic system of the Fleurs radiotelescope and will be required also to engage in design and development work involved in extending the radiotelescope and in improving its performance. He will have the opportunity of using the telescope for radioastronomical observations, and will be expected to make himself familiar with data-reduction procedures.

Salary range: $A10,735-$A11,477 per annum; $US16,000-$US17,000 per annum approximately.

An allowance for fares will be made in the case of an appointee coming from overseas.

Applications, giving details of qualifications and experience and names of referees, by February 21, 1974, to the Registrar, University of Sydney, N.S.W. 2006, Australia, from whom further information about superannuation, etc., may be obtained. Particulars of the post itself should be obtained from Professor W. N. Christiansen in the University. (270)

STERILE PRODUCTS

(SCIENTIFIC AND TECHNICAL SUPPORT)

to work as a member of a team which is responsible for the surveillance of processes used in the manufacture of sterile products in hospitals. All aspects of the work are involved, including the equipment and methods employed. Based in London, but some travel is involved.

Candidates must have a degree in Pharmacy or a Natural Science. Relevant commercial or hospital experience an advantage.

Starting salary within the scale £2,401–£3,237 according to qualifications and experience. Non-contributory pension scheme. Prospects of promotion.

For full details and an application form (to be returned by February 15, 1974), write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone BASINGSTOKE 29222, Ext. 500, or LONDON 01-839 1992 (24-hour answering service). Please quote T/8504.

Department of Health and Social Security

(279)

MONASH UNIVERSITY

MELBOURNE, AUSTRALIA

CHAIR OF PHARMACOLOGY

Applications are invited from suitably qualified medical or science graduates for the position of Professor in Pharmacology in the new Department to be established on the University campus. The new department will be responsible for courses in pharmacology in the Faculties of Medicine and Science at undergraduate and postgraduate levels. Since its inauguration in 1962 the Department of Physiology has been responsible for the teaching of pharmacology, and for some years has offered a major third-year course in pharmacology for students taking the degree of Bachelor of Science, as well as providing basic pharmacology teaching for medical students. Pharmacological research projects have also been pursued by a considerable number of Honours and graduate students in the Department of Physiology.

Additional office and research laboratory space will become available in 1975 for the Department of Pharmacology, and the Department of Physiology will initially provide teaching and research facilities. The Department of Pharmacology will also be closely involved in the development of clinical pharmacology within the Monash Medical School. It is hoped that the Professor of Pharmacology can be appointed during 1974, so that he can participate fully in planning for the development of biomedical sciences during the 1976-78 triennium within which the new department is to become independent.

Salary: $A19,102 per annum ($A20,247 per annum if medically qualified). Superannuation is based on an endowment assurance scheme, the employee and employer contributing 5 per cent and 10 per cent of salary respectively.

Further information and details of application procedure are available from Mr. J. D. Butchart, Academic Registrar, Monash University, Wellington Road, Clayton 3168, Australia, or the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Closing date: March 31, 1974.

The Council reserves the right to make no appointment or to appoint by invitation at any stage.

(273)

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Applications are invited for the post of RESEARCH ASSISTANT to a Biochemist at the Plymouth Laboratory. Candidates should be qualified in Chemistry or Biochemistry. Present research is concerned with the function of enzyme polymorphisms in individual fish and in fish populations. The work involves spectrophotometry, chromatography, electrophoresis and other standard techniques. Candidates should have H.N.C., degree or equivalent. Salary scale on the Civil Service grade of Scientific Officer from £1,318 to £2,177 maximum (under review).

Application forms and further details may be obtained from the Administrative Officer, Marine Biological Association, Citadel Hill, Plymouth PL1 2PB, before January 31, 1974. Quote reference Sup/Bio-Chem. (336)

ROTHAMSTED EXPERIMENTAL STATION

HARPENDEN, HERTS. AL5 2JQ

The Pedology Department has two posts for SCIENTIFIC or HIGHER SCIENTIFIC OFFICERS.

Post A is for research on fundamental aspects of the aggregation and pore structure of clays and clay soils, which requires training in a physical science and preferably experience in a relevant field such as colloid science or surface chemistry.

Post B is to participate in research on organic complexing agents formed in the soil, which preferably requires training and experience in organic and physical chemistry.

Appointments will be in the grade of Scientific Officer £1,590-£2,177, or Higher Scientific Officer £2,076-£2,667 with a non-pensionable salary supplement of 4¼% to offset personal FSSU contributions. Starting salary in accordance with qualifications and experience. The minimum qualification is a good honours degree, and at least two years' post-qualifying experience is required for appointment to the HSO grade. For Post A an appointment as Senior Scientific Officer might be considered for an outstanding candidate with appropriate qualifications and experience.

Applications, naming two referees and quoting reference 215A or B to the Secretary by March 15, 1974. (327)

THE GRASSLAND RESEARCH INSTITUTE

Hurley, Maidenhead, Berkshire

PLANT PHYSIOLOGIST

Applications are invited for a post of Plant Physiologist in the Botany Department of the Institute.

The person appointed will have an important role in a small group, led by the Head of the Department, investigating the physiology of forage crops in the field. The aim is the better understanding of the processes influencing productivity, especially crop photosynthesis, respiration and transpiration.

The group is using a crop enclosure method to measure carbon dioxide and water vapour fluxes, and experience of infra-red gas analysis and other relevant physiological techniques, would be an advantage. However, experience and a sound knowledge of plant physiology in this area and the ability to handle and interpret data are more important.

The Institute is grant aided by the Agricultural Research Council and is an associated Institution of the University of Reading.

Minimum qualifications: HNC, pass degree or equivalent in Botany or allied subject.

The appointment will be made in the grade of Scientific Officer (£1,318-£2,177 p.a.) or Higher Scientific Officer (£2,076-£2,667 p.a.) point of entry depending on qualifications and experience. At least 5 years' post-qualifying experience is required for appointment in the higher grade.

Superannuation Scheme with a non-pensionable allowance of 5½% of salary to offset personal contributions.

Applications to the Secretary by February 18, 1974, giving curriculum vitae and the names of three referees and quoting reference 3/I/3. (293)

AGRICULTURAL RESEARCH COUNCIL

LETCOMBE LABORATORY

A SCIENTIFIC OFFICER is required in the Field Studies Department to assist with measurements of physical characteristics of soils in connection with investigations on the effects of reduced cultivation on root development of crops. Minimum qualifications: pass degree, H.N.C. or the equivalent in an agricultural science. Ability to drive would be an advantage. Starting salary £1,318-£2,177 according to qualifications and experience. Superannuation with a non-pensionable allowance to offset personal contributions.

Application forms and further particulars may be obtained from the Secretary, Agricultural Research Council, Letcombe Laboratory, Wantage, Berkshire. The closing date for application is February 13, 1974.

(281)

UNIVERSITY OF SYDNEY

LECTURESHIP IN THEORETICAL CHEMISTRY

Applications are invited for a fixed-term lectureship in Theoretical Chemistry. The appointment will be made as soon as possible in 1974 and will continue in the first instance until December 31, 1975. Preference will be given to candidates with special interests either in the quantum chemistry of the solid state or in statistical mechanics.

Salary range: $A8,698-$A11,982 p.a.

Applications, including curriculum vitae, list of publications and names of three referees, by January 30, 1974 (will be accepted for a further fourteen days), to Registrar, University of Sydney, NSW 2006, Australia.

Further information available from Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

(269)

LIVERPOOL POLYTECHNIC

SCHOOL OF PHARMACY

RESEARCH ASSISTANT IN ENZYMOLOGY

Applications are invited for the above post with a group investigating the structure and physical/chemical properties of clinically important enzymes and enzyme derivatives.

Candidates should have or expect to obtain a good first degree or equivalent in chemistry, biochemistry, pharmacy or a related area. The successful appointee will be expected to work for a higher degree.

Details available from: Staff Office, Dept 'N', Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool L3 9RH. (309)

THE MEDICAL COLLEGE OF ST. BARTHOLOMEW'S HOSPITAL

WEST SMITHFIELD, LONDON EC1A 7BE

TECHNICIAN required in Biochemistry Department for research into biochemical control mechanisms. Some experience in enzymology and subcellular fractionation useful. Salary up to £2,292+£126 London Allowance according to age and qualifications.

Applications in writing to the Secretary of The Medical College. Further information from Dr J. B. Clark (Tel. 01-253 0661 Ext 22). (323)

AREA LABORATORY KING EDWARD VII HOSPITAL WINDSOR

SENIOR TECHNICIAN required for Department of Microbiology. Laboratory provides a comprehensive service for approximately 1,000 beds in the Windsor, Ascot and Maidenhead areas, and for Out-Patient Clinics and General Practitioners in the area. Expansion of the hospital services anticipated in the future. Hospital close to public termini, within easy access of London by road, and has its own swimming pool.

Applications to Senior Pathologist. (Enquiries to Senior Chief Technician at Windsor 61424.) (354)

PRE- AND POST-DOCTORAL TUMOUR IMMUNOLOGISTS to join the Tumour Biology Group of Westminster Hospital and Medical School, London S.W.1. Work in areas of tumour and lymphocyte culture, cytotoxic and inhibition assays, immunofluorescence, antibody fractionation and chemical coupling, radioimmunoassay of oncofoetal antigens. Regular scientific meetings and clinical contact. Salaries (up to £2,500) and conditions tied to Whitley A scales for Biochemists, according to experience, etc.—Applications to Prof. J. R. Hobbs, Department of Chemical Pathology, Westminster Medical School, London, S.W.1. (247)

RESEARCH DEMONSTRATOR required in Department of Chemistry, University of Reading. Duties will include demonstrating to undergraduate classes in practical chemistry. Good honours degree (or its equivalent) in Chemistry essential and preference will be given to applicants with an interest in Physical Chemistry. The person appointed will be expected to read for a higher degree. 3-year appointment. Salary in the scale £1,047×51—£1,149 p.a.—Apply in writing, quoting Ref TN7, to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH. (322)

UNIVERSITY OF LEICESTER

LECTURESHIP IN THEORETICAL ASTROPHYSICS

Applications are invited for the above post from applicants with interest in one or more of the following areas—planetary physics, infrared astronomy, X-ray astronomy.

Salary according to qualifications and experience on scale £1,929 to £4,548 a year.

Further particulars and application form from the Registrar, to whom applications should be sent by February 28. (345)

UNIVERSITY OF LEICESTER

SENIOR LECTURER IN BIOCHEMISTRY

Applications are invited for the post of Senior Lecturer in the Department of Biochemistry. (Head of Department, Professor H. L. Kornberg, F.R.S.) This is a new post consequent upon the foundation of a Medical School at the University, and the person appointed will be concerned with teaching in the Faculty of Medicine and the Faculty of Science.

Initial salary, dependent on qualifications and experience, within the scale £4,368 to £5,496 a year, with F.S.S.U. membership.

Further particulars from the Registrar, to whom applications should be sent by March 4. (346)

ENVIRONMENTAL CARCINOGENESIS RESEARCH. We need a new member to work in a small group on enzyme induction, its mechanism, and role in cell injury. The main project would be using tissue culture. The suitable person would probably be a biochemistry graduate, or postdoctoral. Initial appointment up to 3 years. A suitable person may be able to register for a PhD and could be graduating in summer to start this Autumn. Salary range £1,500-£2,000 p.a. (Whitley Council Scale).—Enquiries, giving qualifications and any experience, to Dr A. McLean, University College Hospital Medical School, University Street, London WC1E 6JJ. (328)

LECTURES AND COURSES

UNIVERSITY OF BRISTOL

DEPARTMENT OF INORGANIC CHEMISTRY

M.SC.

COURSE IN ANALYTICAL CHEMISTRY

(With Special Reference to Instrumental Methods)

OCTOBER 1974-1975

Applications are invited from persons who hold or expect to be awarded, an appropriate Honours degree or equivalent qualification. The course offers a complete background to the principles and practice of instrumental analytical chemistry.

The aim is to develop a research approach to analytical chemistry and investigation of relevant analytical problems, including pollution studies, will form a major part of the course.

Applications and inquiries should be sent to the Registrar, The University, Senate House, Bristol, as soon as possible. (259)

UNIVERSITY OF LONDON: A lecture entitled "Cell Differentiation in the Cellular Slime Moulds" will be delivered by Dr J. M. Ashworth (Leicester) at 5.30 p.m. on February 4 at University College (Botany Lecture Theatre), Gower Street, W.C.1. Admission free, without ticket—Academic Registrar. (342)

A NATO SUMMER COURSE ON "MOLECULAR AND CELLULAR ASPECTS OF IMMUNOLOGY"

A NATO course in fundamental immunology will be held in CARRY-LE-ROUET (Marseille, France) from September 5 to 14, 1974. Main topics will be: Structure, genetics and biosynthesis of immunoglobulins. Genetics of the immune response. Antigen recognition at the cellular level. Cellular interactions in the immune response.

Information and application forms may be obtained from: Dr Michel Fougereau, Centre de Biologie Moléculaire du CNRS, 31, chemin Joseph Aiguier, 13274 MARSEILLE Cédex 2, France. Deadline for applications: May 1, 1974. (302)

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHYSICAL CHEMISTRY

POSTGRADUATE COURSE IN SURFACE CHEMISTRY AND COLLOIDS

leading to the degree of M.Sc.

Applications to join the eleventh of these twelve-month courses are invited from persons who hold, or expect to gain, an appropriate Honours Degree or equivalent qualification. Studies are arranged to meet the needs of industry and of chemical, biological and environmental research, for graduates trained in modern theory of colloids and surface chemistry and in current experimental methods, including electron microscopy, rheology, light-scattering ultra-centrifugation and other techniques.

The course, beginning on October 3, 1974, is approved by the Science Research Council for tenure of Advanced Course Studentships.

A prospectus giving further details can be obtained by writing to:

Department of Physical Chemistry,
University of Bristol,
Cantock's Close,
Bristol BS8 1TS.

(316)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

R.T.Z. Educational Trust, sponsored by the Rio Tinto-Zinc Corporation Limited, offers awards tenable at the Royal School of Mines for 1974/75 and subsequent academic years.:

R.T.Z. Research Bursary—£695 to £1,000 per annum (according to qualifications and experience), plus College and University fees. Normally for three years. For research connected with applied geochemistry, geophysics, mineral exploration, mining geology, mining, mineral technology or extraction metallurgy. Candidates must hold an appropriate honours degree.

R.T.Z. Advanced Course Bursaries—£695 to £800 (according to qualifications and experience), plus College and University fees. For a postgraduate course of one academic year only in engineering rock mechanics, geophysics, mineral exploration, mineral process design or mining. Candidates must hold an appropriate first degree.

Further particulars and application forms (to be returned by March 31, 1974) from the Registrar, Imperial College, London SW7 2AZ. (340)

UNIVERSITY OF LONDON: A COURSE OF two lectures entitled "Involvement of the Cell Surface in Early Development" will be delivered by Professor A. Monroy (Naples) at 5.30 p.m. on January 28 and 30 at University College (Physiology Lecture Theatre), Gower Street, W.C.1. Admission free, without ticket.—Academic Registrar. (271)

Middlesex Polytechnic

Water Supply and Public Health Engineering

This three-month, full-time course starts in September 1974. It is designed to provide methods and techniques as applied to modern practice in Water Supply and Public Health Engineering.

Scientists, engineers or suitably qualified people engaged in the field of Water Supply, Public Health or Municipal Engineering will be eligible to join the course. **Fee: £15.00.**

For further details write to **The Admissions Office, Middlesex Polytechnic, Ref. N/1/25, PO Box 40, Queensway, Enfield, Middlesex, or phone 804 8131, 805 0892.**

(312)

FELLOWSHIPS AND STUDENTSHIPS

RESEARCH FELLOWSHIPS IN ADVANCED MATERIALS at University of Surrey

Department of Metallurgy and Materials Technology

1. ENERGY FROM FUSION REACTORS

The development of successful Fusion reactors will require materials with improved resistance to neutron irradiation. With this in mind the UKAEA (Culham Laboratory) have sponsored a fellowship to study the formation and growth of helium bubbles in niobium and other potential containment materials. Reactor conditions have been simulated by ion bombardment carried out by the University's Department of Electrical and Electronic Engineering. The Fellow will be required to study bubble behaviour using electron microscopy and to interpret his observations in the light of diffusion kinetics.

The Fellowship will be initially for a period of two years.

2. NEW CUTTING TOOL MATERIALS

A Postdoctoral Fellowship, endowed by Engelhard Industries, is available to continue promising research on new high temperature binder phases for cemented carbides and oxides. An interest in the relation between microstructure and properties, including interfacial energy effects, is central to this research. The appointment will be initially for two years, but is renewable annually.

Salary scales

The Fellows will be appointed at a salary within the lecturer scale (£1,929 to £3,048 per annum), with an annual increment of approximately £165.

Applications with curricula vitae should be sent to: Professor M. B. Waldron, Head of Department of Metallurgy and Materials Technology, University of Surrey, Guildford, Surrey GU2 5XH. (285)

UNIVERSITY OF SHEFFIELD

POSTGRADUATE SCHOOL OF LIBRARIANSHIP AND INFORMATION SCIENCE
SHEFFIELD S10 2TN

INFORMATION SCIENCE STUDENTSHIPS AND POST-DOCTORAL FELLOWSHIPS

Applications are invited from well-qualified graduates for consideration for the award of research studentships and postdoctoral fellowships for research in information science.

Candidates for studentships should normally have, or expect to obtain by October 1974, at least an upper (or equivalent undivided) second-class honours degree in science or technology. Candidates for fellowships should hold, or expect to be awarded by October 1974, a Ph.D. in science or technology. Conditions of award will be similar to those applied by the S.R.C.

Current research in the School includes studies of computer-based information storage and retrieval systems, examination of the microstructure of databases, surveys of data and information use in science and technology, study of national information system design, and investigation of staffing for library and information work.

Preliminary applications should be submitted to Professor W. L. Saunders, Director of the School, by February 14, 1974. Quote Ref. R17/G. (350)

ROTHAMSTED EXPERIMENTAL STATION

HARPENDEN, HERTS. AL5 2JQ

Applications are invited for two Agricultural Research Council postgraduate studentships tenable at Rothamsted Experimental Station. The awards are available for three years from September-October 1974 to students having a first or upper second class honours degree (or those expecting to gain such qualifications in summer 1974) and wishing to undertake research leading to a PhD degree. The studentships may be held in any of the scientific departments of the Station.

The basic tax-free maintenance allowance is £695 for students living in the hostel or lodgings.

Further allowances are payable for postgraduate experience, dependants, or to those who have had previous employment.

Applications with names of two referees by February 20, 1974.

Further information, if required, can be obtained from the Director's Office, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ. (326)

MRC

Medical Research Council

Travelling Fellowships in Cancer 1974-75

The Council invites applications from medically or scientifically qualified workers, normally resident in the UK, of Registrar/Senior Registrar or equivalent academic grade wishing to undertake one year's research training at a suitable centre abroad in clinical or experimental work relating to cancer.

Five of these newly created awards are available at a stipend of £3060 plus overseas allowance. Travel and dependants' allowances are also provided.

The closing date for application is **28 February 1974.** Application forms and further details may be obtained from:

Medical Research Council (Training Awards Group),
20 Park Crescent, London W1N 4AL. Telephone 01-636 5422.

(253)

EAST MALLING RESEARCH STATION

AGRICULTURAL RESEARCH COUNCIL STUDENTSHIP

V. H. BLACKMAN STUDENTSHIP

Studentships tenable at East Malling Research Station for three years from October 1, 1974, for research on the genetics, pathology or physiology of fruit trees.

First or upper-second Honours degree essential.

Further details and application form from Assistant to the Secretary, East Malling Research Station, Maidstone, Kent, ME19 6BJ. (292)

UNIVERSITY OF SURREY

DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING

RESEARCH STUDENTSHIPS

A limited number of Research Studentships are available at the University of Surrey for research in Ion Implantation, Laser Applications and Telecommunications, Control and Computers, and Electronics.

Recent graduates now working in industry, and those about to graduate in 1974, with a good honours degree in Electrical Engineering, Electronics, Physics or Materials Science, are invited to apply to: Professor D. R. Chick, Head of Department, Department of Electronic and Electrical Engineering, University of Surrey, Guildford, Surrey. (331)

QUEEN ELIZABETH COLLEGE

(UNIVERSITY OF LONDON)

CHEMISTRY DEPARTMENT

RESEARCH STUDENTSHIP IN NUCLEAR QUADRUPOLE RESONANCE SPECTROSCOPY

A Research Studentship is available from September/October 1974 for a suitably qualified graduate in chemistry, physics or chemical physics wishing to take a higher degree. The successful candidate could join the following research projects: the study of molecular interactions in solids by means of pure quadrupole resonance at high pressure; quadrupole resonance in group IVA tetrahalides and related compounds; magnetic and quadrupole relaxation studies of molecular motion in crystals. The Department has available a wide range of cw and pulsed radiofrequency spectrometers operating between 0.5 and 400 MHz. The Studentship will be at the S.R.C. rates plus fees.

Interested students are asked to contact Professor J. A. S. Smith (N), Chemistry Department, Queen Elizabeth College, Campden Hill Road, London W8 7AH. (283)

THE ROYAL SOCIETY

ERNEST COOK TRUST RESEARCH FELLOWSHIPS

Applications are invited for three ERNEST COOK TRUST RESEARCH FELLOWSHIPS for environmental studies, including research in pollution control. The appointments are tenable for two years in the first instance from October 1, 1974, and may be renewable for one further year. Stipend range £2,265 × £165 to £2,595 with superannuation benefits.

Applications receivable by March 31, 1974 to be made on forms obtainable, with further details from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG. (330)

THE MEDICAL COLLEGE OF ST. BARTHOLOMEW'S HOSPITAL

WEST SMITHFIELD, LONDON EC1A 7BE

RESEARCH STUDENTS IN BIOCHEMISTRY

Applications are invited from good honours graduates and those expecting to graduate in 1974 for Postgraduate Research Studentships in Biochemistry (£695 p.a.+fees). Successful candidates will be registered for the degree of PhD. Topics will include Immobilized Enzymes, Enzyme Mechanisms, Metabolic Control, Radiation Biochemistry, and Natural Product Chemistry.

Enquiries to Professor E. M. Crook, Biochemistry Department, Charterhouse Square, London EC1M 6BQ. (324)

UNIVERSITY OF WARWICK

POSTDOCTORAL RESEARCH FELLOW IN MOLECULAR BEAM SCATTERING

Applications are invited for an S.R.C. Postdoctoral Research Fellowship in the Department of Molecular Sciences to work on the investigation of four centre ion-molecule reactions. This will include experimental work using an existing crossed molecular beam apparatus at the Thornton Research Centre and the calculation of potential energy surfaces and scattering calculations. Applicants should have experience in high vacuum work, and preferably in molecular beams. The post is tenable from February 1, 1974, for one year in the first instance, with the possibility of renewal for a further two years. The starting salary will be in the range £1,929-£2,388 p.a. with F.S.S.U.

Application forms and further particulars may be obtained from the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No. 24/3Q/74. Completed applications should be returned as soon as possible. (252)

UNIVERSITY OF DUBLIN
Trinity College

Leverhulme European Visiting Fellowship

Trinity College intends to appoint a Leverhulme European Visiting Fellow for the academic year 1974/75 and applications are invited from those outside the British Isles who hold or expect to hold a degree of Doctor of Philosophy or its equivalent from an institution of university status in a Council of Europe member country (except Malta). The Fellowship is tenable in any field of study except Modern European Languages and Literature.

The Fellowship carries a tax free stipend of £1,800 and an additional allowance of £350 may be paid if a Fellow is accompanied by his wife.

Candidates who wish to be considered should send full details of career to date and the names and addresses of two referees to:

The Staff Secretary,
West Theatre,
Trinity College,
DUBLIN 2,

to reach him not later than March 15, 1974.

(307)

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

(UNIVERSITY OF LONDON)

JENNER MEMORIAL STUDENTSHIP

Applications are invited from graduates holding a first- or upper second-class honours degree in a microbiological subject for the Jenner Memorial Studentship tenable in the Institute's Virology Department. The Student will be expected to register with the University of London as a full-time postgraduate student and engage in research for the Ph.D. degree. The Studentship, which is for one year in the first instance, and renewable for a second and third year, is valued at £850 per annum free of income tax.

Applications, with the names of two referees, should be sent to the Secretary, the Lister Institute of Preventive Medicine, Chelsea Bridge Road, London SW1W 8RH. (290)

UNIVERSITY OF WARWICK

POSTDOCTORAL RESEARCH FELLOWSHIPS

Applications are invited for Postdoctoral Research Fellowships in the Department of Physics to investigate the structure and properties of glasses and glass-ceramic materials. Fellowships are available in the following areas: (i) structure and properties of glass surfaces, (ii) crystal nucleation and growth in refractory glasses. The Fellowships will be suitable for Physicists, Metallurgists, or Materials Scientists preferably having previous experience of electron microscopy. Salaries in the range £1,929-£2,388 p.a. with F.S.S.U., depending on age and experience.

Applications in writing (no forms), giving details of previous experience and the names of two referees, should be sent to the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No. 24/4Q/74, by January 31, 1974. (251)

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF CHEMISTRY

RESEARCH STUDENTSHIP

Applications are invited for a Research Studentship for studies in organophosphorus chemistry. The value of the award will be equivalent to an S.R.C. Studentship, and the successful candidate will be able to register for a Ph.D. degree.

Applications, giving a full curriculum vitae and the names of two referees, should be sent as soon as possible to Dr. C. D. Hall, Department of Chemistry, King's College, Strand, London WC2R 2LS, quoting Reference N18/1. (274)

MRC

Medical Research Council

Fellowship Awards

The Council invites applications for the following post-doctoral awards available from October 1974.

Junior Research Fellowships: medical, dental and science graduates (25 Awards).

French Exchange Scholarships: young graduates in medicine or science (2 Awards).

Clinical Research Fellowships: for medical graduates of Registrar or Senior Registrar status; also for dental graduates of similar status (10 Awards)

Anglo-French Clinical Research Scholarships: medical graduates of Registrar status (2 Awards).

The closing date of application for the Junior Research Fellowships and French Exchange Scholarships is 28 February; and for the Clinical Research Fellowships and Anglo-French Clinical Research Scholarships 30 March.

Application forms and further details may be obtained from:

Medical Research Council (Training Awards Group), 20 Park Crescent, London W1N 4AL. Telephone: 01-636 5422. (254)

European Molecular Biology Organization

(EMBO)

Short- and long-term fellowships in molecular biology

The European Molecular Biology Organization intends to award to scientists working in laboratories within the European area both short-term (from a few days to several weeks) and longer-term fellowships for collaborative research or advanced training in molecular biology.

The Short-term Fellowships are to support visits to other laboratories for the purpose of carrying out experiments with special techniques or of other forms of scientific collaboration or advanced training, and especially to support developments arising at short notice.

The Long-term Fellowships will usually be for a period of one year, but applications for renewal will be considered. They will be awarded upon individual application, at the "junior" level to promising young research workers who may then spend prolonged periods in other laboratories working under the guidance of leaders in the field of molecular biology. At the "senior" level, they may be awarded to enable established research workers to gain experience in new approaches and new problems. Upon application by European Institutions fellowships at the "senior" level may also be awarded to specialists who can assist in the initiation and development of research programmes in the sponsoring Institution. Such "sponsored" awards may be made to established workers at all stages of their career beyond the postdoctoral stage.

Application forms and further details may be obtained from Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, 6900 Heidelberg 1, Postfach 2029 (Postfach 1022.40 after July 1, 1974), Germany.

Application for Short-term Fellowships may be made at any time and will be decided upon soon after receipt of the application. Applications for Long-term Fellowships should be made before February 1 for a decision by April 30 and before August 1 for a decision before October 31. Because the selection procedure involves an interview these deadlines for application should be respected.

(X4)

UNIVERSITY OF ABERDEEN

DEPARTMENTAL STUDENTSHIP IN MEDICAL PHYSICS

GRADUATE PHYSICIST required for three years to investigate the phenomenon of emission of light during dissolution of irradiated solids (lyoluminescence) and its possible application in dosimetry of ionizing radiations. Knowledge of chemistry or physical chemistry is an advantage. The candidate appointed will be expected to register for a Ph.D. Post is financed by the National Radiological Protection Board. Value of studentship is £695 per annum with dependants' allowances where appropriate.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by February 16, 1974. (347)

THE NUFFIELD FOUNDATION TRAVELLING FELLOWSHIPS IN MARINE BIOLOGY (TROPICAL AND SUB-TROPICAL)

The Nuffield Foundation offers two Fellowships per annum to enable biologists in U.K. universities to travel overseas (normally for a period of between six and twelve months) to gain scientific experience of inshore marine environments in tropical and sub-tropical regions. Consideration can also be given to applications from physical scientists and from candidates who may be working in institutions other than universities. The primary aim of the scheme is to enliven and to broaden the scope of biology teaching at the tertiary level. The Fellowships are open to men and women holders of U.K. or other Commonwealth university degrees. The value of the awards will be determined on an *ad hoc* basis but will cover fares, subsistence allowances, and contributions to equipment and running expenses.

Applications, for awards to be taken up in 1975, should be submitted by March 31, 1974. Further particulars and forms of application are available from the Nuffield Foundation, Nuffield Lodge, Regent's Park, London NW1 4RS. (264)

GRANTS & SCHOLARSHIPS

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

ROYAL DUTCH SHELL SCHOLARSHIP IN GEOPHYSICS

Open to Honours graduates in Geology (with Mathematics and/or Physics at ancillary level) or in Physics for research in geophysical prospecting as applied to the search for oil. Value £750 per annum plus College fees and approved fieldwork expenses.

Further particulars from the Registrar, Imperial College, London SW7 2AZ.

Closing date March 31, 1974. (257)

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Cannon House, Park Farm Road,
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MISCELLANEOUS

SPECIAL RELATIVITY

Persons who disagree with Einstein's theory of special relativity are invited to write to: Dr P. A. v. Deinse, Adm. v. Gentstr. 9, Utrecht, Netherlands. (308)

SYMPOSIA

International Symposium on 'Approaches to the early Detection of Chemical Toxicity'

September 2-6, 1974

at the University of Surrey, Guildford, Surrey.

Further information from: Dr J. W. Bridges, Department of Biochemistry, University of Surrey, Guildford, Surrey, GU2 5XH. (300)

The Hatfield Polytechnic

One-day Symposium on

"RECENT ADVANCES IN SYSTEMIC FUNGICIDES"

at the Hatfield Polytechnic on Friday, April 26, 1974.

Course fee, including lunch, £10.00.

Full details and programme from Mrs M. Gulliver, Administrative Assistant, Department of Biological Sciences, The Hatfield Polytechnic, P.O. Box 109, Hatfield, Herts AL10 9AB. (337)

Second International Conference on Cyclic AMP

July 8-11, 1974. Vancouver, Canada

Sponsored by the University of British Columbia

PROGRAM

July 8, a.m. T. W. Rall, "Opening Remarks". ADENYLATE CYCLASE: M. Rodbell, "An Allosteric Model for Hormone Action on Adenylate Cyclase"; S. Jard, "ADH Sensitive Kidney Adenylate Cyclase"; G. S. Levey, "Hormonal Activation of Cardiac Adenylate Cyclase"; H. N. Torres, "Adenylate Cyclase in Neurospora Crassa".

July 8, p.m. HORMONE RECEPTOR INTERACTIONS: P. Cuatrecasas, "Insulin and Adrenergic Receptors and the Mechanisms of Modulation of Adenylate and Guanylate Cyclase Activities"; M. Schramm, "Coupling of the Catecholamine Receptor to the Adenylate Cyclase System"; G. D. Aurbach, "Beta Adrenergic Receptors, Cyclic AMP and Ion Transport in the Avian Erythrocyte"; S. Lissitzky, "Thyrotropin-Receptor Interaction and Cyclic AMP-Mediated Effects of the Hormone in Isolated Thyroid Cells in Culture"; M. Sonnenberg, "Growth Hormone Control of Membrane Structure and Function".

July 9, a.m. PHOSPHODIESTERASES: M. M. Appleman, "The Regulation of Cyclic Nucleotide Phosphodiesterase"; S. Kakiuchi, "Regulation of Cyclic Nucleotide Phosphodiesterase Activity in the Nervous System"; J. H. Wang, "Activation of Cyclic Nucleotide Phosphodiesterase by its Protein Activator and by Calcium Ion"; B. Weiss, "Regulation of Multiple Forms of Cyclic Nucleotide Phosphodiesterase".

July 9, p.m. PROTEIN KINASE: J. A. Beavo and E. G. Krebs, "Properties of Rabbit Muscle Protein Kinase"; O. M. Rosen, "Molecular Structure and Characterization of Beef Heart Protein Kinase"; J. D. Corbin, T. R. Soderling, S. L. Keeley and C. R. Park, "Hormonal Regulation of Cyclic AMP-Dependent Protein Kinase in Intact Tissues". CYCLIC GMP: N. D. Goldberg, "The Yin Yang Hypothesis of Biological Control: Opposing Influences of Cyclic GMP and Cyclic AMP in Bidirectionally Regulated Systems"; G. Schultz and J. G. Hardman, "Regulation of Cyclic GMP Levels in Smooth Muscle and Other Tissues"; A. White, "Studies on Guanylate Cyclase Activity in Heart and Lung".

July 10, a.m. CYCLIC AMP IN CARDIOVASCULAR AND SMOOTH MUSCLE FUNCTION: H. Rasmussen, W. Lake and D. B. Goodman, "The Cyclic Nucleotides and Cellular Calcium Metabolism"; W. R. Kukovetz and G. Poch, "Quantitative Relations Between Cyclic AMP and Inotropism as Affected by Stimulators of Adenylate Cyclase and Inhibitors of Phosphodiesterase"; G. Brooker, "Cyclic Nucleotide Fluctuations During the Myocardial Contraction Cycle"; I. Øye and J. B. Osnes, "The Relation Between Cyclic AMP Metabolism and Inotropic Response of Perfused Rat Hearts after Adrenergic Stimulation under Different Conditions"; A. Katz, M. Tada and M. Kirchberger, "Role of Cyclic AMP-Dependent Protein Kinase in the Control of Cardiac Function".

July 10, p.m. E. G. Krause and A. Wollenberger, "Cyclic AMP Enhanced Reversible Protein Phosphorylation and Calcium Binding in Cell Membrane Enriched Preparations from Pig Myocardium"; R. Andersson, "Cyclic AMP—A Regulator Substance in Smooth Muscle Function and Metabolism"; CYCLIC AMP AND INTERMEDIARY METABOLISM: J. H. Exton, "The Role of Cyclic AMP in the Regulation of Hepatic Carbohydrate Metabolism"; R. Ho and E. W. Sutherland, "Cyclic AMP-Mediated Hormone Antagonist in Target Cells"; D. Steinberg, "Hormonal Regulation of Lipase, Phosphorylase and Glycogen Synthase in Adipose Tissue"; U. Schwabe, R. Ebert and H. C. Erbler, "Adenosine Release From Fat Cells: Effect on Cyclic AMP Levels and Hormone Actions".

July 11, a.m. INVOLVEMENT OF CYCLIC NUCLEOTIDES IN NERVE FUNCTION: P. Greengard, "Role of Cyclic Nucleotides in Neuronal Function"; F. Bloom, "The Role of Cyclic Nucleotides in the Central Synaptic Actions of Catecholamines"; J. P. Perkins, "Regulation of Cyclic AMP Content in Normal and Malignant Brain Cells"; M. H. Makman and J. H. Brown, "Cyclic AMP in Retina: Influence of Dopamine and Other Agents and Comparison with Regulation by Biogenic Amines in Other Neuronal Systems".

July 11, p.m. CYCLIC NUCLEOTIDES IN CELL CYCLE, DISEASE AND ENDOCRINES: I. Pastan, G. Johnson and W. Anderson, "Cyclic AMP and Malignant Transformation"; A. G. Gilman, "Hormonal Control of Cyclic AMP Metabolism in Hybrid Somatic Cells"; J. P. McManus, "Role of Cyclic Nucleotides and Calcium in the Positive Control of Cell Proliferation"; J. J. Voorhees, "Imbalanced Cyclic AMP-Cyclic GMP Levels in Psoriasis"; J. N. Singh and N. S. Dhalla, "Adenylate Cyclase Activation due to Allogenic Lymphoid Cell Interactions in Graft-Versus-Host Disease"; H. Bourne, K. Melmon and Y. Weinstein, "Control of Leukocyte Function by Receptors for Vasoactive Hormones"; F. Labrie, "Role of Cyclic AMP in the Action of Hypothalamic Releasing Hormones in the Anterior Pituitary Gland"; G. A. Robison, "Concluding Remarks".

CONTRIBUTED PAPERS

The program will also feature contributed papers (BRIEF COMMUNICATIONS) which can cover a broad range of topics related to cyclic nucleotide metabolism and function. For instructions for preparing abstracts for brief communications, for registration forms and information on living accommodation, write to Dr. George I. Drummond, Department of Pharmacology, School of Medicine, University of British Columbia, Vancouver 8, Canada. (604) 228-2703. Proceedings of the conference will be published as Vol. 5 of Advances in Cyclic Nucleotide Research (Raven Press).

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Synopses of the British Fauna (New Series) No. 5

BRITISH SEA SPIDERS

Arthropoda : Pycnogonida

Keys and Notes for the Identification of the Species

P. E. King

January 1974, approx. 80 pp., softcover

0.12.407950.4

This work, the first complete key to the British Pycnogonida, will fill an important position in the literature of a subject which previously has been almost ignored.

The species listed have been checked by the most up-to-date methods of numerical taxonomy along with stereoscan electron microscope observations. The keys, illustrated by simple, clear figures, should enable anyone with a minimum knowledge of the arthropods to make accurate identifications in the laboratory or the field.

Physical Chemistry of Melts in Metallurgy Volume 2

F. D. Richardson FRS

January 1974, approx. 260 pp., approx. £5.50/$15.50

0.12.587902.4

Outlining our present knowledge concerning the chemistry of liquid melts, salts, mattes and slags, this two volume publication forms a selective work aimed at highlighting those systems which exemplify important principles or which are of industrial importance. This is the first work which brings together our knowledge of structural kinetic and thermodynamic properties of melts important in metallurgy, and considers the kinetics of reactions between them in terms of chemical, electrochemical cad mass transfer phenomena.

Contents

Thermodynamic Properties 11: Slags and mattes. Heterogeneous equilibria and free energy diagrams.

Kinetics: Chemical kinetics. Transport kinetics. Interfacial phenomena. Reactions between liquid metals and gases. Reactions between liquid metals and slags or salts.

The Cell in Medical Science

Volume I

F. Beck and J. B. Lloyd

January 1974, approx. 400 pp., approx. £8.00/$22.50

0.12.084201.7

The recent dramatic advances in cell biology consequent upon the introduction of sophisticated cytological and biochemical techniques have enlarged the field to the point where no publication can set out to provide a comprehensive coverage of the whole of the subject. The editors have therefore selected a number of basic topics, each of which are treated in depth by acknowledged experts, and combined morphological, physiological and biochemical details in each section. The first volume of this four-volume work deals with the generalised cell unit, and devotes chapters to its principal organelles.

Techniques of Physics Series No. 2

Low Energy Electron Diffraction

The Theory and its Application to Determination of Surface Structure

J. B. Pendry

January 1974, approx. 430 pp., approx. £9.00/$25.25

0.12.550550.7

This book aims to give a coherent account of the most recent theory of LEED plus enough description of experiments to set the theory in context.

Contents

Introduction. Scattering processes for low energy electrons. Principles of diffraction at $T=0^{\circ}\mathrm{K}$. Schemes of calculation. Perturbative methods and related techniques. Temperature effects. Applications of LEED to surface structure analysis. Appendices. References. Author index. Subject index.

Biology of Plant Litter Decomposition Volume 2

Edited by C. H. Dickinson and G. J. F. Pugh

January 1974, xii+610 pp. £10.50

0.12.215002.3

Contents

The Organisms

E. Eklund and *H. G. Gyllenberg:* Bacteria. *M. Goodfellow* and *T. Cross:* Actinomycetes. *G. J. F. Pugh:* Terrestrial fungi. *E. B. G. Jones:* Aquatic fungi: freshwater and marine. *J. D. Stout:* Protozoa. *D. C. Twinn:* Nematodes. *J. R. Lofty:* Oligochaetes. *D. J. L. Harding* and *R. A. Stuttard:* Microarthropods. *C. A. Edwards:* Macroarthropods. *C. F. Mason:* Mollusca. *M. Ladle:* Aquatic crustacea.

The Environment

S. T. Williams and *T. R. G. Gray:* Decomposition of litter on the soil surface. *C. H. Dickinson:* Decomposition of litter in soil. *L. G. Willoughby:* Decomposition of litter in fresh water. *E. J. Perkins:* Decomposition of litter in the Marine environment. *R. S. Forbes:* Decomposition of agricultural crop debris. *K. R. Gray* and *A. J. Biddlestone:* Decomposition of urban waste.

Organism Index. Author Index. Subject Index.

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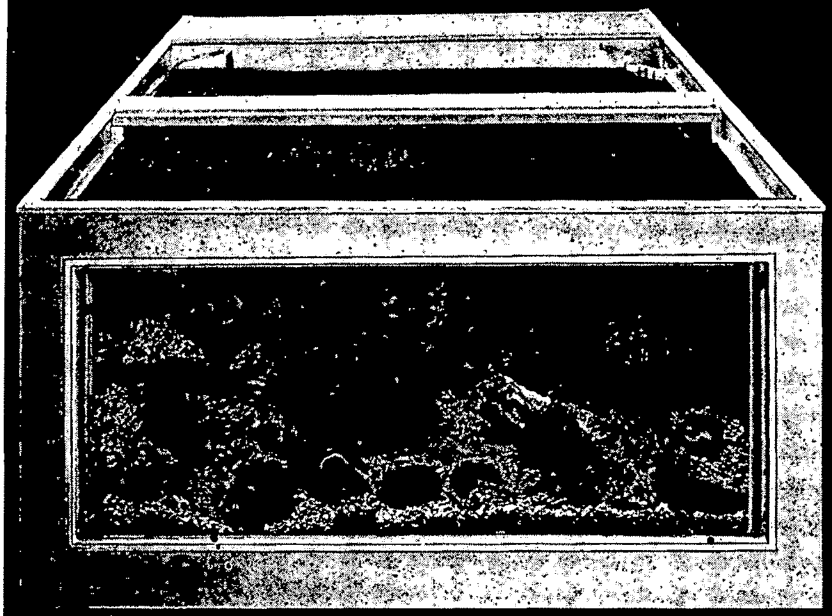

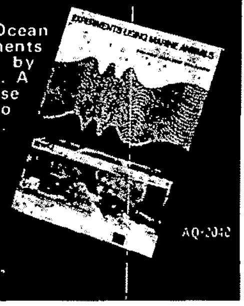

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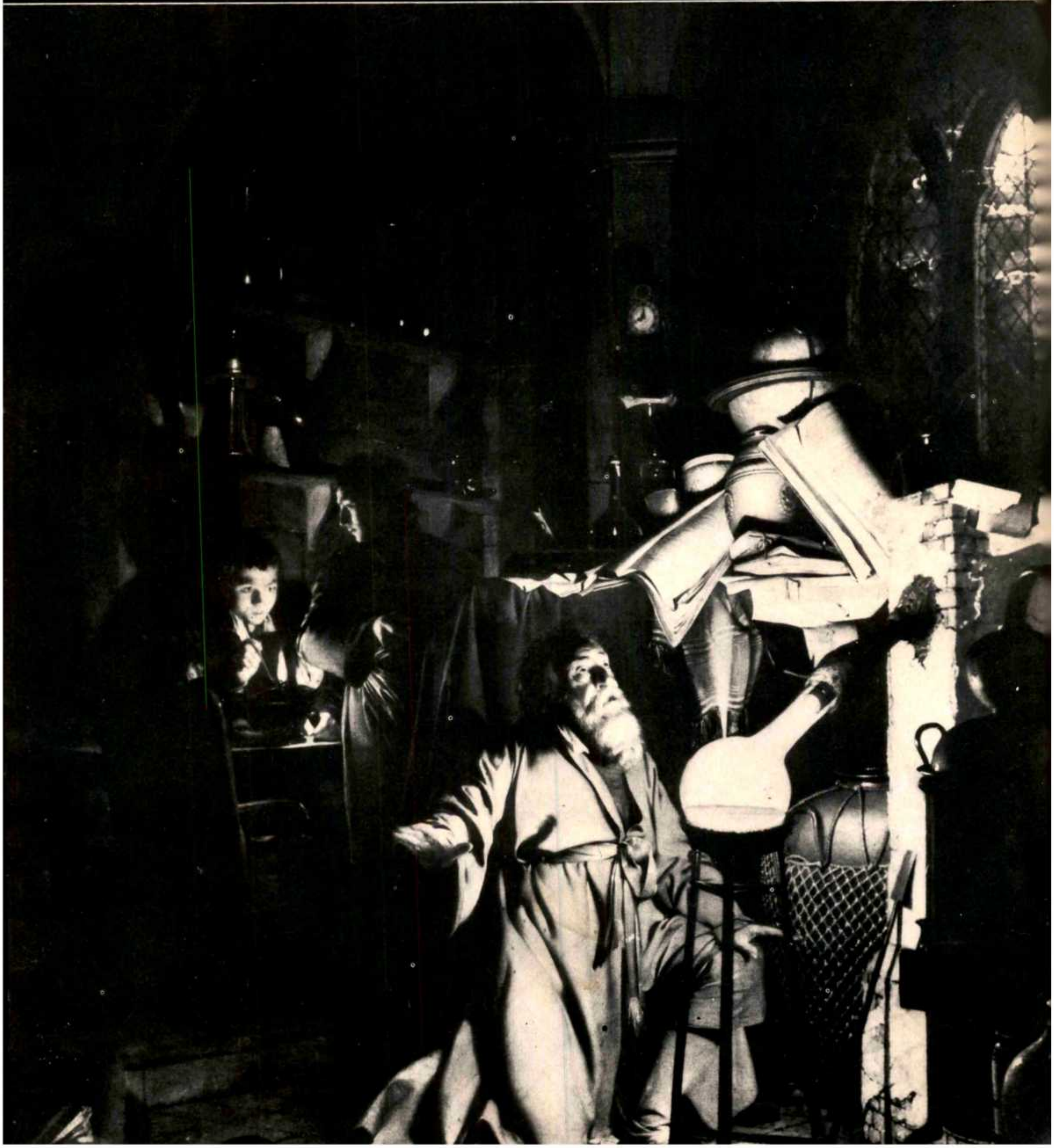

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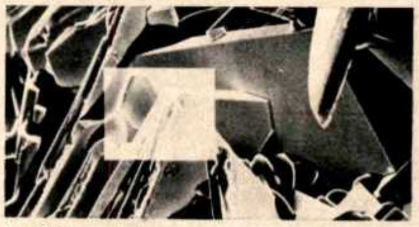

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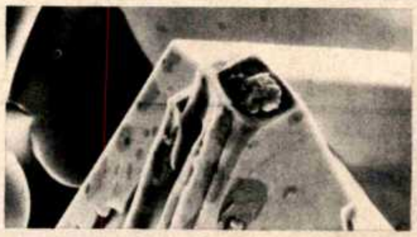

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'The Alchymist in Search of the Philosopher's Stone discovers Phosphorus' by Joseph Wright of Derby, 1734–97 (Derby Museums and Art Gallery). See also page 245.

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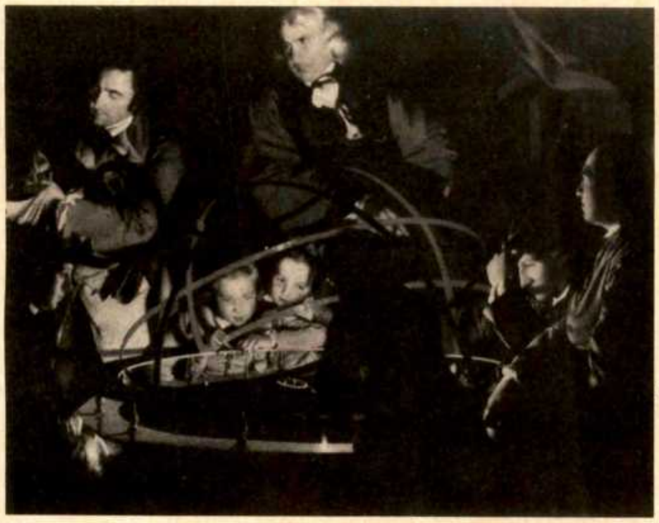

Wright of Derby

One of the greater pleasures of boyhood was the prospect of five or six weeks of holiday completely devoid of thoughts about school. It was in the second or third week that long days of two-a-side football games with the other boys in the street would begin to pall. Simultaneously the semi-annual visit to the dentist loomed large and one bleak morning I would be despatched on the fourpenny-return busride into Derby to be told the results of six months of neglect. The only consolation was spending the rest of the day in the Public Library.

Derby was no delicate maiden of a town, no Salisbury, Ely or Hereford. Instead it had industrial prides such as a large locomotive factory; it built aeroplane engines and, so they said, had the best bus station in the country. This was more relevant stuff to a nine-year-old. The Public Library abutted on to the Museum and Art Gallery and the whole of what might now be called an arts complex, but then was not called anything in particular, was intended to serve a very industrial town.

The library books, among which most of the rest of my post-dental day was spent, were witness to what had made the town great. Shelf after shelf of books on stern industrial subjects, devoted to the self-improvement of the populace. There was a limited number of volumes of a lighter character so after an hour or two of flipping through books explaining conjuring tricks or giving tips on home photography, my attention would wander to the art gallery next door. It was not exactly stacked with crowd-pulling impressionists nor do I recall seeing any Picassos there. It was really a one-man show, and to my mind, a two-painting show. The man was Joseph Wright (1734–97) and the two paintings The Alchymist, on our cover this week, and The Orrery, on this page.

Wright was a contemporary of Reynolds, Gainsborough and Romney and, like them, he made his living from portrait and landscape painting. Unique among painters of that period, or almost any other period for that matter, he was, however, fascinated by the scientific experiments and industrial inventions that were beginning to stir the country's consciousness. He had discovered a rich seam and it is our loss that he did not exploit it more frequently; the Air Pump in the Tate Gallery, London is the only other full-scale painting of his on a scientific theme.

Wright used light to the full, particularly the effect of low illumination from candles—indeed it was the sight of *Nature's* staff similarly illuminated which reminded us of the paintings. He was also positively photographic in his detail. The characters are all either local practitioners of science and alchemy or, as the audience, identifiable schoolmasters, businessmen or their children. His observations of scientific instruments seem to have the same precision, and it is reasonable to assume that even the bizarre phosphorescent scene has its basis firmly in actual observation by the artist.

The pictures tell a fascinating story not only of eighteenth century experiments in the name of science but of a time when science was probably more uniformly spread through the country. Each town had its own vigour; the pull of the metropolis and the universities for science barely existed.

The effect of Wright's paintings was always overpowering. The bus journey home to tea was filled with the afterglow of confronting this view of the past. How many other Derby boys had their scientific awakenings from such an unusual source?

100 years ago

NATURE

We have received the first Annual Report of the "Haileybury Natural Science Society." It contains preliminary lists of the fauna and flora of the place, together with observations on the meteorology of the locality, and a humorous description of an experimental dinner at which the principal dish consisted of esculent snails which had been specially fed and fattened for the purpose by certain members of the Society. It need scarcely be added, that the repast amply rewarded the members for their generous devotion to the cause of Science.

From *Nature*, 9, 274, February 5, 1874.

Why plant physiology suffers in India

The penurious state of Indian research in plant physiology is described by the President of the International Association for Plant Physiology, Professor H. G. Burstrom of Lunds University, Sweden.

At a meeting of the Indian Society for Plant Physiology at Simla in 1971 an international committee was set up to examine Indian research in plant physiology*. There has been a general feeling that Indian research in plant physiology is not meeting the demands that could be made on it, and that a better understanding of the subject is now essential for rational exploitation of cultivated plants.

A report was submitted in July 1973[1] which probably reflects conditions in several other developing countries. Many details are of national concern only, but some major problems cannot be solved without international help.

Indian research in plant physiology is mainly done in the botany departments of sixty-five universities (including agricultural universities) and in ten independent research institutions. The committee sent a questionnaire to 150 plant physiologists throughout India, and fifty replies were received from forty-four institutions of different sizes and quality.

About 70% of laboratories report a lack of funds. Restrictions on the availability of foreign exchange make the purchase of chemicals and equipment manufactured abroad difficult and exorbitant prices are demanded. Few chemicals and little equipment are produced in India, and usually when they are, the quality is poor, and often unsuitable for precision work. About half of the laboratories have difficulties in purchasing essential chemicals. Too often scientists have to ask for chemicals as gifts from colleagues abroad. Only about 25% of laboratories have no problems of foreign exchange; these are the institutions maintained by the Government of India.

The University Grants Commission has given financial help to some large universities such as Delhi, Calcutta, and Madras to develop centres of advanced study in certain disciplines of botany, but none so far in plant physiology. In the universities it forms only part of botany, and appropriations go to botany departments. Only a small fraction of the money reaches the plant physiology laboratory, unless the head of the botany department himself is a physiologist. Most of the research in these laboratories is supported by grants for specific projects, amounting to a few thousand rupees a year, quite often not enough to sustain the project itself. Several laboratories have profited considerably from generous grants from the United States PL 480 funds. So there are two extremes—the well equipped and the poorly nourished—and most are poorly nourished.

More than 60% of laboratories consider that lack of instruments limits the choice of research subjects; about two-thirds have difficulty maintaining current research due to lack of equipment—both small and large. Only three or four laboratories consider themselves well equipped. Foreign industries selling instruments in India provide only sparse maintenance facilities. Few institutions have their own workshops, and instructions on instrumentation and instrument maintenance are hard to come by.

There is thus an inclination towards less expensive research, for example, simple experiments involving only the application of hormones and other chemicals to growing plants. This has serious implications. Although research in more expensive fields is essential due to their application in economic plant husbandry, such studies are rarely undertaken.

Few laboratories have the climate-conditioned growth chambers and glass houses essential for basic research. The ambitious work by Indian physiologists under field conditions lacks reproducibility and the results are liable to several interpretations. In a vast country such as India, with extremes of climate, growth chambers are all the more necessary; although expensive, they save time and manpower. For genuine field trials, however, adequate land space is necessary. Unfortunately even the agricultural research institutions allot to physiological research too small a part of the land available.

In the special physiology units, wherever they exist, staff strength is inadequate. Teams of people with training in analytical methods are usually lacking, and technical assistance is often inadequate. Few scholarships are available for physiological research. In several places there is no provision for research for master's and doctor's degrees in plant physiology.

Library facilities are usually poor. Of the major, exclusively plant physiological journals, *Plant Physiology, Physiologia Plantarum, Plant and Cell Physiology, Journal of Experimental Botany*, and *Planta* are subscribed to by only about 60, 40, 30, 20, and 15% respectively.

In most new institutions back volumes of journals are not available. Even the so-called affluent institutions cannot purchase them for lack of currency. Many institutions do not receive abstracting journals, and the need for an information bank which would supply on request published physiological information is keenly felt. Setting up an information bank, or even strengthening an existing unit, may be expensive, but would be worthwhile.

Many young physiologists complain of the absence of a congenial human environment, lack of encouragement, incentives and appreciation of good work done, lack of drive from superiors, want of opportunities for exchange of ideas, and too many bureaucratic procedures and administrative bottle-necks. The committee has noted cases where the head of a well-equipped laboratory thinks these are no problems, but his subordinates feel otherwise. This human element should not be overlooked, but material deficiencies dominate the picture.

There is a demand from some quarters for the establishment of a Central Institute of Plant Physiology on the pattern of the national laboratories. Probably it will be more useful if five good plant physiology laboratories in north, south, east, west, and central India—one in each zone—are developed into well-equipped Centres of Advanced Study in Plant Physiology, corresponding to the centres set up in other branches of botany. These new centres should also look into the difficulties of the smaller laboratories in the region and help them as far as possible. Workers in adjacent areas should be welcome to work in these laboratories. Association with larger research groups and the organisation of conferences will help the exchange of ideas and partly neutralise the feeling of isolation and frustration expressed by many scientists. Invitations to foreign experts and visits to foreign countries by Indian workers to a larger extent than now will also be useful. This cannot be realised without help from UGC, ICAR, and international fund-giving organisations.

Nevertheless, improvements must begin at the level of the basic training of students. If only advanced research were encouraged—and this is more attractive than scientific education—a gap may develop between the qualifications of graduated scientists and the requirements necessary for advanced research.

---

* Its members were Dr S. Boon-Long, Ministry of Agriculture, Bangkok; Dr R. G. Lincoln, Long Beach, California; Professor S. P. Sen, University of Kalyani, India; and Professor H. G. Burström (chairman).

Plant physiological subjects of immediate importance are, for example, mineral nutrition, water balance for irrigation and hardiness problems, hormones as regulators of translocation, growth and development, nitrogen metabolism, and protein production from both old and new sources. Even the highly theoretical topic of photosynthesis has gained in practical importance after the discovery of the special anatomically-linked mode of photosynthesis in tropical and subtropical plant species, responsible for their high productivity. There is also an urgent need for a study of the physiological parameters of crop yield in the Indian region. Too little or no research is done in these fields in India.

An appreciation of the importance of plant physiological research for crop improvement, and closer collaboration between physiology and genetics with adequate support from the policy-making bodies, will help to improve the situation. But the problem is so vast that international support is needed.

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[1] Burström, H. G., Boon-Long, S., Lincoln, R. G., and Sen, S. P., *The present state of plant physiological research in India. A report of its problems* (stencil), 152 pp. Appendix i–iii (Indian Society for Plant Physiology, 1973).

international news

Civil Service scientists take action on pay

John Hall

Memories of the dear dead days of the Aldermaston demos by the Campaign for Nuclear Disarmament (CND) were stirred by the prospect of government scientists broadcasting messages of protest at the gates of their own establishment. An Aldermaston gates meeting was one of the disruptive moves planned as part of a national work to rule started by the scientists' section of the Institution of Professional Civil Servants (IPCS), who are demanding a restoration of salary parity with the Civil Services's administration and executive classes (AEC). The scientists at the Atomic Weapons Research Establishment showed signs of their militant mood as long ago as December, when the announcement of a pay research settlement for the AEC prompted them to fire off a telegram to the civil service department in the following terms:

"Well deserved AEC award rubs salt into wounds already inflicted by CSD. Aldermaston scientists demand justice. Unfair comparisons will bring disruptive action."

A meeting of more than 400 scientists who sent the message decided on "uncooperative" action in January unless a satisfactory pay settlement was made. The mention of unfair comparisons referred to methods of determining what the basis for an increase should be—a problematical question, since, the scientists hold, there are no valid industrial parallels which might help the Pay Board to come to a decision.

The "uncooperative" action was started on January 21, when the IPCS's 20,000 scientific members embarked on a fifteen-point programme of protest actions which included meetings during working hours, half-day strikes and overtime ban. Among the more devious strategies which the disaffected scientists planned was for all suitably qualified staff to apply for every job vacancy advertised in the Ministry of Defence's weekly lists. This would virtually jam the entire machinery of making appointments in key research establishments.

At the end of the first week of 'uncooperative" action at Aldermaston, an IPCS spokesman there declared with satisfaction that scientific work at the establishment had almost come to a stand-still. This was due in large part to the introduction of a scheme requiring all communications within the establishment and with the outside world to be conducted by letter rather than by telephone. No research programme proceeds at white heat when colleagues who are able to wave to each other through their windows elect to exchange views by means of a protracted correspondence.

Industrial action was precipitated by news that the Pay Board, already criticised for being tardy over the scientists' claim, announced that it would not be ready to report before march at the earliest. This news was the straw which broke the camel's back, for it extended the disparity (and the frustration it has generated) that has existed between the pay of scientists and executive civil servants since 1971.

Although there was a correlation between the two branches before that date, a difficulty in assessing criteria for determining a scientist's pay allowed the administrators to jump ahead while the scientists' case was chewed over. Now, on entry as a Scientific Officer, a graduate can expect to earn £1,300 to £2,200, while his counterpart, the Executive Officer, can expect £1,700 to £2,600. Higher up the scale, the Principal Scientific Officer receives £3,500 to £4,600, while a principal in the administrative group expects £4,100 to £5,400.

In further support of their demands, the scientists point out that between January 1971 and November 1973 the retail price index rose 27.1%, the weekly wage index rose by 39.9%, and the pay of government scientists rose by between 19.4% (in the highest grade) and 23.9% (in the lowest grade). And that included a 7% stage three entitlement.

Nixon announces energy budget

Colin Norman, Washington

In a highly unusual move, President Nixon last week treated the American public to a detailed preview of his Administration's forthcoming budget request for energy research and development in the 1975 fiscal year. The figures, contained in a fact sheet distributed by the White House along with this year's first Presidential energy message to Congress (there were at least three such messages last year), herald a veritable bonanza for research and development aimed at increasing production and use of coal.

The total expenditure proposed for all energy research and development is $1,810 million, an increase of 81% compared with this year. All areas of research get a look in, but some are more favoured than others—nuclear fission takes pride of place, for example, carrying off some 40% of the proposed funds, and large increases are earmarked for the development of technologies to control pollution from power plants (a programme whose chief purpose is to enable coal containing high sulphur contamination to be burned to generate electricity).

The spending proposals are the fruit of a frenzied planning process which began in September, when President Nixon asked Dr Dixie Lee Ray, Chairman of the Atomic Energy Commission, to prepare a five-year plan for energy research and development. Her report (see *Nature*, **246**, 370; 1973), delivered to the White House early in December, forms the basis of the Administration's budget request, although some of her recommendations have been altered rather drastically (and surprisingly) by the Office of Management and Budget.

Although direct comparisons between Dr Ray's proposals and the budget figures are difficult to make, there are some notable differences in priority. First, the Administration's budget proposals have heaped considerably more money into coal research than Ray recommended—$577.7 million (including environmental control technology) against $405 million. Second, Ray's suggestion that $166.2 million should be spent on developing technology for more efficient use and storage of energy, has been reduced; the budget request for such research and development comes out at $115.7 million. Finally, the total expenditure envisaged by the Administration's budget proposals is a good deal higher than that recommended by Ray—$1,810 million compared with $1,570 million.

In line with the goals of President Nixon's much-publicised Project Independence, which are essentially to make the United States self-sufficient in energy as quickly as possible, the spending proposals entail vast concentration of resources in areas which are likely to have a quick payoff, although the reduction in the proposals for conservation research are surprising. Dr Ray in her report that by 1985, conservation efforts are likely to save as much as 7 million barrels of oil imports per day. She thus recommended that $1,440 million should be spent on such research and development over the next five years; the Administration's budget proposals recommend only $700 million during that period of time. Even at that level, however, the outlays next year would be nearly 80% greater than this year's expenditures.

As for longer term efforts, the budget proposals entail expenditures of $168.6 million on thermonuclear fusion research, a figure which is somewhat misleading since it includes military research on laser fusion. The Administration is in fact suggesting that $102.3 million should be spent on controlled thermonuclear research, compared with Ray's recommendation of $135.0 million, and last year's outlay of $57.0 million.

Very large increases are suggested for solar and geothermal research, however, with the former getting $50 million and the latter $44.7 million—increases of 262% and 310% respecively over this year's outlays.

When Dr Ray's report was published in December, it ran into some criticism because it recommended too little money for supporting programmes in basic research, health effects and manpower development. The Administration clearly took heed of such criticisms, for it has added $216 million to the budgets for such activities next year.

Ray's report also ran into criticism because it paid too little attention to exploiting the technology that should flow from the research programme. In this case, however, the Administration did not respond to the critics, for it reduced by half—to $50 million—a proposed programme for joint government-industry financing of demonstration plants for coal liquefaction and gasification.

The budget proposals announced last week are far from being money in the bank. For one thing, they must be approved by Congress as part of the usual appropriations procedure (although in election year, members of Congress are unlikely to reduce the requests). For another, the figures give no details of where the money will come from, or which areas of research (if any) may suffer. That may become clear on February 4, when the rest of the budget will be published.

TABLE 1 Federal energy research and development programme (millions of dollars)

Programme area	Program level fiscal year 1973	1974	1975	change 74–75 (per cent)	estimated total FY 1975–79
1. Conservation	32.2	65.0	115.7	78	700
2. Oil, gas, shale development	18.7	19.1	41.8	119	400
3. Coal production and utilization	85.1	164.4	426.7	260	2,900
4. Environmental Control	38.4	65.5	178.5	173	800
5. Nuclear fission	406.5	530.5	724.7	37	4,000
6. Nuclear fusion	74.8	101.1	168.6	67	1,600
7. Other	16.5	53.5	154.5	189	900
solar	4.0	13.8	50.0	262	
geothermal	4.4	10.9	44.7	318	
total	672.2	999.1	1810.5	81	

Boost for coal research in UK

John Hall

In Britain the energy crisis has wrought a virtual overnight transformation in the role of the National Coal Board (NCB) from caretaker of a sad and declining industry to great white hope for tomorrow's power. Now the board plans to ask the government to finance a £400 million investment programme, which will include an element of more than £25 million for research. The government is not expected to think twice about providing the money.

At the same time, the NCB has advised Lord Carrington, Secretary of State for Energy, to be cautious about committing the country to an expanded nuclear power programme and has asked to meet the Government in order to urge "a change of philosophy".

According to Mr Leslie Grainger, board member for research, Britain has enough coal reserves to maintain current output levels for 100 years or more. The research programme, he said, is directed at making more efficient use of coal than is achieved by current methods of conversion to electricity and the sum requested is realistically costed, as as compared with German and United States budgets of £125 million and $2,900 million.

The NCB is already well advanced in laboratory scale work on the conversion of coal into other forms of energy and expects to have economically competitive plants in operation by the 1980s. The immediate need, therefore, is for hardware and engineering specifications deriving from research experience and pilot plants started within the next year or two could be working productively within the next five years.

A demonstration fluidised combustion boiler generating 20 MW and costing something like £2 million, would probably be the first operational project to emerge from the NCB's new programme. It could be working by 1976 and providing data for a 100 MW plant. A liquefaction plant, scheduled for completion the following year, would produce a fuel, equivalent to a petroleum feedstock, by adding hydrogen to coal dissolved in an oily solvent. An output of 10 tons a day is already a feasible proposition, according to the NCB. At about the same time a start could be made on a pilot gasification unit producing gas of low calorific value suitable for use with gas turbines. A number of plants might profitably operate on the same site, turning out fuels, chemical feedstocks and energy, and depending mutually on the by-products of each process.

The government's biggest commitment to energy research at the moment is, of course, in the area of nuclear power. In a written reply to a House of Commons question on the amount of investment in research into sources of energy alternative to oil Mr Peter Emery, Under Secretary of State for Energy, said that the United Kingdom Atomic Energy Authority is spending £42.5 million in the current year on research and development of nuclear fission reactors. The major part of this effort is devoted to the prototype fast reactor, which is expected to achieve "a significant level of power output" early this year.

The Central Electricity Generating Board is also carrying out research in the nuclear power field and Britain has an interest in nuclear fission research as an EEC partner. Another European energy venture, is a research programme on hydrogen from water and on solar energy, estimated to have cost the community a total of £0.9 million in 1973.

Spies in Canada's research council

from David Spurgeon, Ottawa

MANY Canadians, including scientists and Members of Parliament, were startled in January by the allegation contained in a television broadcast that their country's National Research Council (NRC) has for many years been serving as a cover for secret intelligence gathering.

Although the NRC unit involved—the Communications Branch—has been in existence since 1940, few Ottawa officials seemed to be aware of its existence or its mission. The director-general of information services in the Department of National Defence—a brigadier-general who has served with the department for some 30 years—told an inquirer that before the television program he had never heard of it.

Those who do know of its mission have been sworn to secrecy. Its director, N. K. O'Neill, refused to give a reporter information on its budget and staff, or indeed to comment on anything concerning it. When asked to whom he reported, he replied that he had been spending all that morning trying to find out. Questioners seeking information from the NRC were directed to the Prime Minister's office (where a spokeman simply quoted from Mr Trudeau's comments on January 11 in the House of Commons. The Prime Minister acknowledged that Canada "has always collected what information was available to it in its territory", but said it had never to his knowledge "engaged in any espionage abroad in the sense that we have not been looking for information in an undercover way in any other country". And he would not confirm the allegations made in the television program.

The program, aired by the Canadian Broadcasting Corporation (a Crown corporation funded by the federal government), claimed the NRC branch works hand-in-hand with the United States Central Intelligence Agency through the CIA's representative at the United States embassy in Ottawa.

It monitors and records radio traffic received through listening posts in the Arctic and elsewhere, and analyzes this and other intelligence gathered by the Royal Canadian Mounted Police and the armed forces. Encoded messages sent from foreign embassies in the Canadian capital are also monitored. The results of this work are shared with allies such as the United States and Britain.

Regardless of the accuracy of the details, it is apparent that the NRC's Communications Branch is in fact engaged in highly secret work of a nature more usually associated with defence or security agencies than with national research laboratories. Inquiries reveal that the branch came into existence during the second world war, when the NRC seemed the obvious place to go for help because of its scientists' expertise and contacts.

What seems strange is that the work was not moved after the war, either to the Defence Department or the newly-formed Defence Research Board. One reason seems to be that NRC simply provided too good a cover, and since moving the branch would only draw attention to it, those responsible felt it best to leave it where it was. It also seems strange that the matter has never before been raised publicly, because the branch is situated in its own building surrounded by a high wire fence in plain view in southwest Ottawa (albeit far removed from other NRC buildings), and is listed in the government telephone directory.

Now that the branch has surfaced, its work is clearly an embarrassment to the NRC. Those in a position to know say attempts have been made by NRC for years to get rid of it. They also say NRC has contributed nothing to its policy, and that the president and council know little or nothing about it.

The importance of the affair for Canadian scientists is the effect it could have abroad on the image of the country's national laboratories. The NRC has been one of Canada's chief instruments of international science policy and its representative on many international scientific agencies. To be tarred with the CIA's brush will not help its reputation.

There are national implications too. As Douglas Fisher, former Member of Parliament and newspaper columnist put it in the Toronto *Sun*: "Highly placed people noting Watergate's excesses, get concerned. Could the Communications Branch, NRC, get out of hand? . . . it's expenditures and work are never openly questioned or examined".

The questions raised by the television program may, however, lead to changes. David Lewis, leader of the New Democratic Party, which has held the balance of power in the minority government, asked in the Commons: "In view of the fact that the NRC obviously is not the appropriate agency for such an operation . . . may I ask the Prime Minister whether the government would consider taking (it) out . . . and placing it in a more appropriate department. . . ?" To which Mr Trudeau replied that he would consider the suggestion.

Are PWRS good for Britain?

John Hall

WITH the British government hotly tipped to fall, and fall soon, for the attractions of a United States light water nuclear reactor in an attempt to stave off a threatened energy gap, there has been a lot of fast talking in London about the pros and cons of available nuclear options. Both Sir Arnold Weinstock of GEC and Lord Aldington, Chairman of the National Nuclear Corporations, have owned that they favour the American pressurised water reactor (PWR) as the best option for securing cheap power in the 1980s. Their evidence for this preference has not been received as gospel truth, either by the House of Commons Select Committee to which it was presented, or by interested parties which included the Institution of Professional Civil Servants (IPCS) and the Trades Union Congress (TUC).

The energy resources sub-committee of the Select Committee on Science and Technology started hearings on the choice of reactor after information leaked from the Central Electricity Generating Board (CEGB) last autumn to the effect that its chairman, Mr. Arthur Hawkins, had a strong preference for the United States equipment as a quick and cheap answer to Britain's short term power problems. The current programme of advanced gas colled reactors is at least £500 million over budget, is running late by up to six years and is, according to Mr. Hawkins, a catastrophe which should not be repeated.

The select committee, alarmed at the prospect of a *fait accompli* in this most delicate and expensive area of technological development, immediately concerned itself with an inquiry into the choices before the Government. Its air of scepticism in the face of pro-PWR witnesses like Sir Arnold and Lord Aldington has been undisguised, and when towards the end of January an imminent ministerial decision was rumoured, the committee made an abrupt move (with more witnesses expected to testify) to present its report. That report is expected to say, in effect, that the case for buying American is not proven.

Chief among the factors weighing heavily against the American technology was the questionable safety factor of the PWR, with the spectre of catastrophic pressure vessel failure being raised by government scientists (as part of the IPCS case for buying British) and, less graphically if no less earnestly, by Sir Alan Cottrell, Chief Scientific Adviser to the British Government. The civil servants' man pointed out that a failure of a pressure vessel would let loose on "a trusting populace" a nuclear blast with a force equivalent to that of several Saturn rockets. And Sir Alan, in a memorandum to the select committee, indicated that rupture of the steel vessels involved would not admit the inclusion of any fail-safe device and that the vessels might even be subject to failure caused by corrosion or thermal shock induced by an influx of cold water in a loss-of-coolant accident.

Not that the light water reators have been unduly maligned on their service records; the suggestion was simply that, although they might be tried and tested on a worldwide scale for smallish systems, insufficient work has gone into safety proving PWRs of the size that the British power system requires. The chief selling points of the PWRs, their cheapness and ready availability, also began to look rather sick when the Nuclear Inspectorate let it be known that it had no details of the proposed designs and that it might take two years to approve the designs when it had them. Such a delay, and the cost of any modifications which the inspectorate might think necessary, take away a few of the attractions of the American system as a short term solution.

A further pro-PWR voice, Sir John Hill, Chairman of the United Kingdom Atomic Energy Authority, hedged his bet by opting for middle term British reactors of the steam generating heavy water variety, with fast breeders following. For immediate needs the American system is probably the best bet, he considered, although he held back at endorsing the CEGB's estimate of a requirement of, initially, 18 LWRs and ultimately, twice that number. The chairman of the select committee, Mr. Arthur Palmer, made a fairly mild assessment of Sir John's attitude when he said he found him "surprisingly indulgent" to the CEGB's United States line.

The TUC, while reserving its right to make a hard and fast decision, appeared distinctly unnerved by the prospect of imported nuclear hardware and asked for meetings with the CEGB. Apart from expressing concern over the safety (or absence of safety) in PWRS, the TUC's fuel and power committee was said to be worried about the effects of an overseas purchase on the long term development of British nuclear alternatives, and also that the CEGB's estimates of nuclear power requirements would fall short of actual demand. It is bound by a Congress resolution that manufacture and construction for an expanding nuclear energy programme should "wherever possible" be British. The committee has received strong representations from trades unionists that this is one of those occasions on which it is distinctly possible.

Perhaps the strongest protest against the CEGB's plan was made by the IPCS which claims 100,000 members, including 8,000 who work in nuclear energy. Like every other anti-PWR voice in the shouting match. The civil servants were aghast at the safety risks involved in buying a reactor design which was introduced only a year ago and is not scheduled to be translated into an on-line operational plant before 1981. Apparently, unresolved safety problems relating to the effectiveness of the emergency core cooling systems and the continued integrity of the steel pressure vessels over a prolonged period have already caused the American Physical Society to ask for an inquiry.

But the crux of the IPCS case was economic. Its line was that a country with a nightmarish balance of payments problem was not doing itself much good if, at one stroke, it managed to run up a heavy bill in licensing fees for a project which is also calculated to hinder Britain's prospects as an exporter of nuclear power technology. The IPCS reckoned that Lord Aldington's estimate of 100,000 to 200,000 for the first stage licensing fees to Westinghouse was a super-optimistic calculation; it would be more realistic, in its book, to talk rather in terms of millions of pounds for the first one or two reactors.

Its proposal, the IPCS confided to Lord Carrington, Secretary of State for Energy, is to press on with AGRs as the major reactor type used for electrical power in Britain and to build at least one steam generating heavy water reactor (SGHWR) in order to open up an export market in developing countries.

After the AGRs, the aim should be to move as quickly as possible to HTRs as the basis for the next cycle of power stations, according to the IPCS proposal.

For Britain, the evidence of Sir Alan Cottrell seemed to emerge from the welter of conflicting information as straight talk from an upright citizen with no particular axe to grind. His conclusion about the possibility of the gradual growth of a small crack caused by ageing and corrosion in high stress regions of a PWR might be taken for the general view on the programme as a whole: "it needs further investigation".

Short notes

Continuous microtron

A PROTOTYPE continuous microtron (a cyclic accelerator in which the electrons are accelerated by microwaves) has been constructed in Obninsk, as a result of combined research by a number of institutions throughout the Soviet Union. Until now, it has proved possible to operate microtron accelerators in a pulsed mode only, with a relatively low repetition frequency (typical figures are a pulse duration of 1 μ s and a repetition frequency 100 Hz).

The new continuous microtron is designed to accelerate electrons to energies of 7.5 to 9 MeV with a current beam of up to 2 mA. Thus the power of the beam on the target will be of the order of 15 to 18 kW, considerably greater than with any other type of microtron.

Seismology and power stations

ONE spin-off of the current programme of energy expansion in the Soviet Union has been a survey, carried out by the Academy of Sciences of the Armenian SSR, into seismic activity in the mountains, valleys and ravines of Armenia, with special reference to possible sites for power stations.

The project had two purposes. On the one hand, a team of archaeologists investigated the evidence of earthquake damage in the past; for example, knowledge of the resistance of structures to earthquakes, and the inclination of surviving architectural relics, provides data on the periodicity of earthquakes in the area and the resistance of various local materials to seismic damage. At the same time, a team under Academician A. G. Nazarov studied three reference sites, taking borehole electrical readings to study the vertical profile of the crust.

news and views

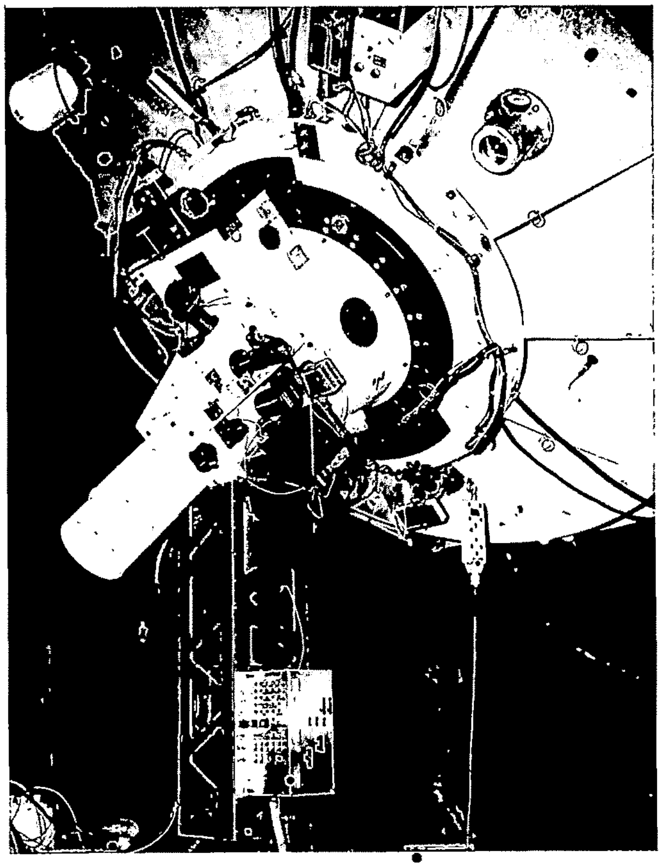
Impact of TV sensors on astronomical research

For many decades, optical astronomers have relied largely on photographic materials to record their data. The method is operationally simple, allows large quantities of information to be stored and, perhaps psychologically important, presents those data in a form similar to that perceived directly by the eye. Although the quantum efficiency ($\lesssim 1\%$) is intrinsically low, values close to those of the best photoemissive surfaces (approximately 20%) can be obtained by using the photographic emulsion in conjunction with an image intensifier tube. The main disadvantages of photographic recording are small dynamic range, non-linear response and difficulty in deriving accurate quantitative data from the plate. These deficiencies become major limitations in, for example, the study of objects which are fainter than the sky and for which background subtraction is therefore necessary. Single channel detectors with the required properties (such as the photomultiplier tube) have, of course, been used for some time, but recent developments in low light level television-type sensors (see *Proc. Conference on Astronomical Observations with Television Type Sensors* (ed. by J. W. Glaspey, and G. A. H. Walker), Institute of Astronomy and Space Science, University of British Columbia, 1973) have raised the exciting prospect of a linear multi-channel replacement for the photographic work horse. In addition to this primary role of collecting data, television systems are now being used to aid the astronomer in actually operating the telescope. With the aid of a TV monitor, faint objects can be located within a field of stars and placed in the instrument aperture with far greater efficiency than before.

TV sensors come in many shapes and sizes. All, however, have an imaging target which is subsequently scanned by an electron beam. For some tubes, like the silicon target vidicon, the photon-sensitive surface doubles as the storage target. In others, a conventional photo-cathode plus electron imaging optics are used to intensify the image created on the storage target. Most systems currently in use monitor the analog signal output of such tubes. For the range of light levels encountered in astronomy, there have been accompanying problems of linearity, dynamic range, and photometric accuracy. Nonetheless, useful astronomical results ranging from abundance studies of the lunar surface (McCord and Bosel, in Glaspey and Walker, 137) through star cluster photometry (Westphal *ibid.*, 127) to spectroscopy of quasars (Lowrance *et al., Astrophys. J.*, **171**, 233; 1972) have been obtained with these devices. In an attempt to avoid some of the difficulties of the storage analog mode, an image intensifier tube may be placed in front of the TV sensor and the latter then used to count individual photon events (Boksenberg and Burgess; Gilbert; Morton, in Glaspey and Walker, 21, 83, 193). The figure shows one such system operating at the Cassegrain focus of the Steward Observatory 90″ telescope. This approach is clearly more accurate and linear but has encountered problems of rather low maximum count rates and of data handling. It thus seems that although TV sensor systems offer great potential in astronomical research, this potential remains largely to be realised. Only in the area of star field acquisition has TV technology so far produced a strong impact on astronomical studies.

It is inevitable that when a new technological advance occurs, wildly enthusiastic claims should be made about its capabilities. In this particular case, one of the wilder claims has been that TV detectors will somehow make large telescopes obsolete. This is nonsense. Increased detective quantum efficiency will increase the effectiveness of large and small telescopes in the same proportion. This gain was made years ago with conventional image tubes and has nothing particularly to do with TV sensors. Certainly for a given observation time smaller telescopes can now do things previously only possible on larger instruments. Far more important, of course, is the fact that large telescopes are able to tackle problems that were not possible before. Even with an ideal detector, many of the important problems of, for example, cosmology are photon limited, especially when competing against the sky background noise. The only way to increase the number of photons is to increase the telescope size whether it be a single large mirror or an array of smaller ones working as a single unit. The latter concept is the basis of the Multiple Mirror Telescope now being constructed by the University of Arizona and the Smithsonian Astrophysical Observatory on Mt Hopkins. The reason for not constructing single mirror telescopes of aperture much in excess of 150″ is simply one of cost effectiveness; it has nothing to do with TV sensors.

It should also be remembered that detector technology is advancing very rapidly. At least two systems are already in



A photon-counting TV detector mounted on the Cassegrain spectrograph of the Steward Observatory 90″ telescope. The detector package hangs beneath the white spectrograph housing.

operation which, for certain applications, seem more promising than TV sensors. These are the phosphor storage scanner constructed at the Lick Observatory (Robinson and Wampler, *Publs. astr. Soc. Pacif.*, **84**, 497; 1972) and the Digicon pulse counting image tube system developed at the University of California, San Diego (Beaver and McIlwain, *Rev. scient. Instrum.*, **42**, 1321; 1971). Looming on the horizon are self-scanned, semiconductor diode arrays which ultimately promise sensitive reliable imaging in a small package. No doubt all of these systems will seem old-fashioned in three years time. The important thing is for astronomers to take advantage now of instruments available now.

P.S.
G.G.

Behaviour of hybrid primates

ONE of the most interesting questions which faces workers in the area of behaviour of higher animals is the degree to which the behaviour patterns are genetically determined and the degree to which they are determined by the ecological conditions of the species. That has been a particular problem in the study of primates because one of the most important adaptations of these animals is the tremendous flexibility of their behaviour in different circumstances. That has led some people to suggest that there is little or no genetic basis for behavioural differences between species. But the existence of a zone of hybridisation between two closely related primate species in central Ethiopia has provided a unique opportunity for examining the genetic and ecological factors which together determine certain aspects of the behaviour of the two species. These have been the subject of a field study by Nagel, who has now published his findings (*Folia primatol.*, **19**, 104; 1973).

Hybrids between anubis (*Papio anubis*) and hamadryas baboons (*P. hamadryas*) are found at the western end of the Danakil depression (Awash Falls). Although this is not the only hybrid zone to have been studied in primates, the anubis-hamadryas zone is unusual because of the marked differences between both the social systems and the habitats of the two species. The hamadryas baboon (also called the desert baboon) occurs mostly in arid or semi-arid regions in Ethiopia (Kummer, *The Social Organization of the Hamadryas Baboon*, University of Chicago Press, Chicago, 1968). Its social system follows the harem pattern, in which each adult male is permanently associated with one or more adult female. The harems are loosely associated in 'bands' which sometimes forage together; and two or more bands, comprising a 'group', sleep together on one of the sleeping rocks in their desert habitat. Kummer (*Primate Societies*, Aldine, 1971) suggests that the three levels of social organisation of the hamadryas are an adaptation to the extreme conditions of the environment. The smallest social unit, the harem, provides a mechanism for foraging in small groups on the widely dispersed food resources while still guaranteeing that each foraging group has an adult male for protection from predators. The larger aggregations are the means by which the harems can come together to utilise more concentrated resources such as sleeping rocks and water sources.

The anubis baboons have the more common type of social structure, in which there are no permanent associations between males and females and the matings are more or less promiscuous, subject to some bias on the basis of the dominance ranking of the adult males. The anubis baboons are found throughout East Africa and in parts of Ethiopia in savannah habitats. The groups are permanent except for occasional movements of adult males from one group to another, and sleep and forage as a single unit, the members usually remaining within visual or at least vocal contact with each other.

The hybrid zone is in an arid region which is much more like the usual hamadryas habitat than the usual anubis habitat. It does not coincide with any obvious ecological barrier and in view of evidence for its persistence in more or less the same place for at least the past 60 years, and possibly longer, Nagel has attempted to identify some of the factors responsible for this stability.

Narrow zones of hybridsation between geographic races have been studied in a variety of species, often under the heading of 'area effects'. It is thought that a reduction in the fitness of the hybrid individuals could result in the maintenance of a stable narrow zone. Even in such well studied species as the European land snail (*Cepeae nemoralis* and *C. hortensis*), however, the actual mechanism of the reduction in fitness has not been found. Nagel finds that the reduction in gene flow and the lowering of the fitness of the hybrids can both be accounted for by the differences in the social systems of the two species. Hamadryas males were seen to 'kidnap' older juvenile anubis females in the same way as they would hamadryas juvenile females in order to start their own harems. The abducted females seemed to learn to cooperate with the herding behaviour of the males and fit into the harems, and were seen with hybrid infants. On the other hand, anubis adult males could not mate with hamadryas females because they did not attempt to herd the females and establish permanent bonds with them. Nor could hamadryas males conform to the anubis social system. In consequence, there is a one-way gene flow between the anubis and hamadryas populations, resulting entirely from the absorption of anubis females into the haradryas harems.

The hybrid zone was identified as the region in which there were harems with hamadryas or hybrid males and anubis, hamadryas or hybrid females. It is important that the hybrid males did not enter the anubis groups: the hybrid males,

according to Nagel, were not as good at herding their females as were the pure hamadryas. This reduction in the fitness of the hybrid males might be sufficient to account for the narrowness of the hybrid zone, for which it can be argued theoretically that only a relatively small reduction in the fitness of the hybrids is needed (Slatkin, *Genetics*, in the press). Because anubis and hybrid males had the same opportunities to learn the herding behaviour as the pure hamadryas, Nagel concludes that the tendency to herd females and form permanent harems is at least in part genetically determined.

Another salient factor, in Nagel's view, is the desirability of small foraging units in the habitat occupied by the hybrid population. Even in the pure anubis group, there was the tendency to break up into foraging groups which would be apart for some part of the day and occasionally sleep apart. The anubis social system was not flexible enough, however, to take full advantage of the small foraging groups and the anubis range was thus limited by the inability to displace the hamadryas from the arid region. This failure to adapt once more suggests a genetic component. On the other hand, the anubis tendency to sleep in trees and the hamadryas tendency to sleep in rocks seemed to have no genetic component, each species apparently utilising the most accessible and the safest (from predators) sleeping sites available.

The existence of the hybrid zone incidentally raises questions about the classification of the genus *Papio*. The situation in the taxonomy of primates is already so complex that the common names of the primates are frequently more useful than the scientific names. Although five species of African baboons have been recognised (*P. hamadryas, P. anubis, P. cynocephalus, P. papio* and *P. ursisus*) there have been observations of free hybridisation between *P. cynocephalus* and *P. anubis* in Kenya (Maples, *Am. J. phys. Anthorp.*, 36; 1972) and now between the anubis and the hamadryas in at least two locations in Ethiopia. The conditions begin to argue for reclassification of *Papio* as a single species or a 'super-species' with different geographical races. Unless emotional attachment to the labelling of fellow primates prevents it, there will have to be yet another change in the nomenclature. Certainly greater differences in morphology of geographic races exist in other species. M.S.

New method of predicting earthquakes

from our Geomagnetism Correspondent

AFTER many years of pessimism in which limited progress towards a solution seemed hardly to justify the vast effort of data collection, earthquake prediction studies seem to have entered a new, more hopeful phase. Although it is less than a year since Whitcomb *et al.* (*Science*, **180**, 632; 1973) reported their discovery of V_p/V_s ratio changes preceding the San Fernando earthquake of 1971 and proposed a physical model to explain them, Gupta (*Science*, **182**, 1129; 1973) has come up with a quite different premonitory phenomenon which involves seismic velocities. As a method of predicting earthquakes it seems to be potentially as useful as the earlier discovery and can be explained in terms of the same physical model, but it has the additional practical advantage that it can be applied using a single three-component seismograph.

The phenomenon itself involves the splitting of S waves and is not, as such, a new discovery. Both laboratory and theoretical studies (see, for example, Nur and Simmons, *J. geophys. Res.*, **74**, 6667; 1969) have shown that when non-hydrostatic stress is applied to a rock containing cracks, an anisotropy is induced in the S wave velocity causing the S wave to split into two distinct components with different velocities. By analogy with double refraction behaviour of light waves in stressed glass, the seismic version has come to be known as "acoustic double refraction" and its magnitude, expressed as the difference between the two component velocities, increases as the deviatoric stress increases.

The question now arises, of course, as to whether the phenomenon investigated theoretically and found experimentally in the laboratory can be observed in the field. Gupta has demonstrated that it can, at least in central Nevada where the faulting is primarily related to crustal extension. The main events for which Gupta sought premonitory (and subsequent) S wave splitting were two earthquakes of magnitude 4.0 and 3.9 with epicentres in the Slate Mountain and Mina regions, respectively; and the sources of the observed S waves were the foreshocks and aftershocks of these events. For each main event the difference (Δt) in the arrival times between the two S wave components gradually increased to a maximum and then decreased again towards its original value; and in each case the main shock took place just after the peak Δt had been reached. In numerical terms, the peak values of Δt lay in the range 0.7–0.9 s, which compares with the apparently normal value of about 0.2 s. The peak S wave splitting was thus small (about 2.5% of the total travel time). On the other hand, it was just about the greatest likely to be observed at this location because S wave velocity anisotropy is greatest along the direction of crustal extension and the source-receiver line was in each case within about 20° of this direction.

On observational grounds alone, therefore, frequent monitoring of Δt values would seem to be a promising prediction technique—and a particularly attractive one in that, in principle, a given area can be monitored using just one three-component seismograph. On a theoretical level, Gupta claims that the observed Δt variation is consistent with pre-earthquake stress accumulation according to the elastic rebound theory, and he has already (*Bull. seism. Soc. Am.*, **63**, 1157; 1973) shown it to be explicable in terms of the dilatancy-fluid saturation model used to explain premonitory V_p/V_s changes. The time scales of the two precursors are also apparently comparable. Whitcomb *et al.* found, for example, that the precursor time interval for a magnitude 4.0 event is about 25 days. For Gupta's magnitude 4.0 shock Δt began to rise from its "normal" value of 0.2 s just over 30 days before the shock.

Helical hypodermic

from a Correspondent

BACTERIOPHAGE, literally 'bacteria eater', is the term used to describe viruses which attack bacteria. Some of these are very elaborate, for example, T phage, and have morphologically distinct head and tail assemblies. The phage fastens itself tail first onto the surface of its victim, injects its genetic material through the tail into the interior of the bacterium, and thereby assumes the biochemical machinery of the host and rapidly spawns new phage progeny. The key process of infection is therefore the penetration of the bacterial cell wall by the phage tail. It is powered by a contraction of the phage tail sheath from a long narrow tubular structure to a short fat one. Since the sheath is a relatively simple structure comprising a regular helical arrangement of protein subunits, it provides an excellent system for the study of an elementary contractile process which is not only of intrinsic interest but which may also serve as a useful model in the analysis of contraction in more advanced organisms. In one important respect, however, the sheath's activity differs from that of other contractile systems, such as flagella and muscle, since the contraction occurs once and for all, that is, it is not reversible.

In the case of T-even phages, the presence of sheaths in the extended and contracted states has often been observed in the electron microscope, and structural studies of the two forms have led to models for the movement of the protein subunits during the contraction. In order to test these models it was necessary to try to follow the process of contraction itself, to catch the phage 'in the act'. In a recent issue of the *Journal of Molecular Biology* (**80**, 613; 1973) Moody shows how this may be done. From a chance observation he has discovered that treatment with formaldehyde at *p*H 8 can produce partially contracted sheaths, and from a number of electron micrographs he is able to erect a gallery of views of the sheath at progresssively shorter lengths. It is important here to consider whether the chemical treatment produces structures which correspond to those of the living organism, and Moody points out that where partially contracted sheaths can be produced by ultraviolet light (which is generally less effective), the views can be fitted into the same sequence. Thus two different artificial methods produce identical results, which are likely therefore to be substantially the same as in the physiological process. Certainly the formaldehyde causes a smooth and progressive transition into a contracted sheath which appears identical to that normally observed.

The sheath has 144 identical subunits. Both extended and contracted forms show helical lines of subunits, and, in addition, cross striations, which are annuli of subunits. The simplest type of contraction mechanism is one in which all subunits move in the same way (though not necessarily simultaneously) and with this restriction each annulus remains intact during contraction. Different types of subunit movement can in principle be distinguished by the characteristically different appearances of the surface lattice of the partially contracted sheath that they produce. From models elegantly drawn with the aid of a computer and a Calcomp plotter, Moody shows that certain 'transitional' helices are formed as the structure shortens which eventually give rise to a well defined set of helices of short pitch observed in the contracted sheath. Different types of subunit movement produce transitional helices of markedly different appearances, and it is not difficult therefore to correlate the models and micrographs and make the selection. A visual comparison is substantiated by detailed measurements of selected parameters in the micrographs, which show that a smooth transition takes place between one of the prominent helical families in the extended sheath and the short-pitch helices in the contracted. The subunits do not all move at once. The contraction commences at the base plate and is transmitted by successive annuli, so that a wave of contraction moves towards the head of the phage.

In order to understand more fully how each subunit moves during the contraction, Moody considers the spatial relationships between subunits in the extended and contracted sheaths and shows how the bonding pattern between adjacent subunits may be rearranged during the transition. In regions between subunits where stain and thus solvent is observed to penetrate, there are unlikely to be strong intersubunit bonds. This reduces the number of parameters that need to be considered. During contraction the bonds between the units of each annulus may undergo only minor realignments (of the type, for example, observed in haemoglobin during the oxy/deoxy transition), but larger movements are required along the 'transitional' helices. The larger movements must involve sliding of the subunits over each other and probably also changes of the subunit shape.

Large movements are permissible in an irreversible process like the sheath contraction, in contrast with the small movements that are a prerequisite of the reversible processes involved in other contractile systems. Moody proposes that the extended form of the sheath is unstable. The formation of this unstable configuration is either dictated by the structure of the base plate, which forms a template for the sheath assembly, or it may be stabilised by the core, which has the same helical symmetry. In either case, it apparently requires only a small stimulus for the stored energy of the sheath to be released. Since the stimulus, arising at the base plate, is transmitted by the wave of contraction itself, the sheath has the properties of both excitation and contraction, and may be considered a primitive 'nerve-muscle'.

Vole aggression and population density

from our Animal Ecology Correspondent

AMONG the theories purportively governing rodent populations, Chitty proposed one to explain the return to 'normal' densities from 'high' densities during cycles of population (*Proc. ecol. Soc. Aust.*, **2**, 51; 1967). The theory held that the return is brought about by a balanced polymorphism of aggressive behaviour phenotypes shifting in favour of enhanced aggression when population increases. This phenotype adversely affects reproduction and juvenile survival. Population decline is the inevitable result.

There is nothing basically different between a 3 or 4 year cyclical pattern of increase and decrease and the normal annual pattern. The same sort of pressures are put on the same resources, so it would be logical to assume that the mechanisms which return population density from high to low are, in each pattern, the same. That assumption calls for a demonstration of the existence of an annual cycle of aggressiveness. This has now been done by Turner and Iverson working with *Microtus pennsylvanicus* (*Ecology*, **54**, 967; 1973), and their study joins those of Sadlier (*J. Anim. Ecol.*, **34**, 331; 1965), Healey (*Ecology*, **48**, 377; 1967) and Krebs (*Ecology*, **51**, 34; 1970) as evidence supporting Chitty's theory.

Turner and Iverson's study was a mixture of field and laboratory observations conducted on a population of *M. pennsylvanicus* occupying an old field habitat at Pinawa, Manitoba. Demographic data were collected from a trapping grid during fortnightly trapping periods each of 2–4 nights' duration. The study lasted 17 months. The level of aggression in the population was determined by observing, for 10 min periods, the number of 'aggressive acts' performed in an arena by two male combatants taken from a representative sample of the wild population. The main acts of aggression used in the analysis were 'threat' (aggressive lunge of one vole towards another), 'vocalisation' (squeaking audible to the observer), 'mutual upright' (violent bipedal boxing) and 'fighting'.

Several attempts have been made in the past to convey a comprehensive picture of aggression. Sadlier and Healey, working with *Peromyscus*, used a sum of six aggressive acts and the total number of threats and chases respectively. Krebs used the number of attacks per approach in *M. ochrogaster* and *M. pennsylvanicus*. Turner and Iverson are the first workers to regard vocalisation as an aggressive act. Vocalisations occur very frequently and any straightforward numerical analysis would over-emphasise their importance. They weighted the four aggressive acts equally. These were then subjected to principal component analysis, and an index of aggression for each encounter was produced by multiplying the weighted values of each act by the factor determined by the principal component analysis. The index showed that aggression increased in frequency at the onset of reproduction and decreased with cessation of breeding. The increases and decreases were statistically significant.

When various population parameters were examined it was found that home ranges in the breeding season were significantly larger that those in the nonbreeding season. Home ranges of overwintered voles in the breeding season were significantly larger than those of young born in the breeding period. Perhaps surprisingly, there was no significant relationship between an individual's level of aggression and its home range size. Adult voles were more aggressive than young and resident home range holders were more aggressive than non-residents.

All the data collected point to the complicity of rapid change in aggression at the time of most rapid change in population size. Eventual intrinsic control of population could be caused either by a strict spacing behaviour or by an interference with breeding performance in the manner suggested by Chitty. Either way, aggression could be seen to play an important part.

Lidiker's long term work on an island population of *M. californicus* (*Ecol. Monogr.*, **43**, 271; 1973), reported in these columns a few weeks ago (see *Nature*, **246**, 380; 1973), suggested an alternative type of natural control in which no one factor was markedly more important than several others. Quite obviously much more detailed study of the complex processes of rodent population stability is necessary if the answer (if, indeed, there is to be just one answer to a problem which occurs wherever rodents exist) is to be found. One thing is clear. Dynamic stability, or the lack of it, is the result of behavioural and physiological change, not the cause of it.

High spin states of nuclei

from our Nuclear Theory Correspondent

REACTIONS between heavy ions are proving valuable tools for the investigation of high spin states of nuclei at high excitation energies. Heavy ions readily produce such states because they bring energy to the compound nucleus without high velocities; for example, 100 MeV can be brought in by a ^{10}B nucleus or by a single nucleon but the velocity of the nucleon would be much higher than that of the ion and so there would be a greater likelihood of direct reactions or of disintegration of the target nucleus. Furthermore, if the collision takes place non-centrally the compound nucleus is given a large angular momentum, so there is an enhanced probability of exciting high spin states.

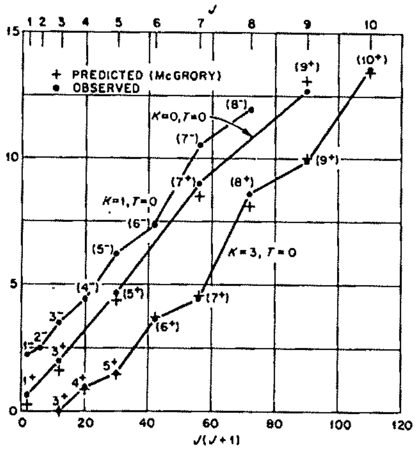
This selectivity of heavy ion reactions is important because at moderately high excitation energies there is often a high density of nuclear states. Only a very few of these have high spins and a heavy ion measurement at relatively low resolution is able to pick them out easily whereas a less selective reaction would require much higher resolution to show them with the same degree of clarity.

The spin of a particular state may be obtained by comparing the angular distribution of the reaction leading to it with the predictions of the statistical theory of nuclear reactions due to Hauser and Feshbach. This theory predicts that the angular distribution is symmetric about 90° in the centre-of-mass system and gives cross sections that depend quite critically in shape and magnitude on the assumed spin of the final state. The identification of the reaction mechanism may be further checked by studying the characteristic fluctuations in intensity of the emerging particles as a function of incident energy.

This method of studying high spin states has recently been used by Gomez del Campo, Ford, Robinson, Stelson, McGrory and Thornton (*Phys. Lett.*, **46B**, 180; 1973) to determine the energies and spins of rotational states in ^{22}Na by the ^{10}B(^{16}O, α)^{22}Na reaction.

The angular distributions of the alpha particles emitted at energies corresponding to several states of ^{22}Na were found to be very well fitted by the Hauser-Feshbach statistical model calculations, confirming both the assigned spins and the assumed character of the reaction.

These high spin states in ^{22}Na are members of rotational bands and the dependence of their energies on J(J + 1) is shown in the figure. It is clear that the bands can be followed to high spin values, and that there are systematic deviations from the J(J + 1) proportionality. Further confirmation of the spin assignments is provided by the energies given by shell-model calculation, and these are also included in the figure.



Excitation energies of states in three rotational bands in ^{22}Na as a function of J(J+1). The dots refer to the measured energies and the crosses to shell model calculations.

This example shows the advantages of heavy ion reactions for studying high spin states, and it is likely that this method will be extensively used in the future.

Surface changes in transformed cells

from a Correspondent

THE idea that the composition of the cell surface controls to some extent the orderly proliferation of cells in culture now occupies a central position in cell biology. The classical experiments of Aub and his colleagues and later of Burger and Sachs and their collaborators demonstrated surface changes in a particularly simple manner by agglutination with certain lectins. The susceptibility of cells to agglutination could be correlated with the growth characteristics of these cells in culture. Subsequent arguments concerning the basic mechanisms behind the increased agglutination of transformed cells compared with non-transformed cells as induced by concanavalin A or wheat germ agglutinin have left the problem still not completely resolved. But there is general agreement that the changes are essentially small quantitative differences in the amount of receptors at the cell surface able to form attachments with multivalent lectin ligands, or in the topographical distribution of these sites.

Clearly, receptors for wheat germ agglutinin, for instance, are present on normal as well as transformed cells and some progress has been made in their characterisation from both cell types (Jansons and Burger, *Biochim. biophys. Acta*, **291**, 127; 1973). The demonstration of clear qualitative change in the surface membrane composition of cells that can be correlated to growth control would therefore be important. What may turn out to be just such a change in cells and their virus induced transformants is described by Hynes (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3170; 1973).

What Hynes has done is to treat clones of the hamster fibroblast line NIL, which show normal behaviour in culture, with lactoperoxidase. In this process external proteins on the surface membrane of the cells are iodinated with

^{125}I and can be identified by radioautography after separation of membrane components by SDS-polyacrylamide gel electrophoresis. Several iodinated proteins are identified in the normal NIL cells. If cells pretreated with lactoperoxidase are subsequently exposed briefly to a low concentration of trypsin, all but one (of molecular weight about 130,000) of the iodinated proteins bands disappear from the radioautograph of the polyacrylamide gel. Clearly therefore these components have been cleaved from the cell surface, whereas one iodinated protein is resistant to proteolysis.

When virally transformed cells were examined a different result was obtained. In these cells an iodinated band of molecular weight greater than about 200,000 is missing from the radioautographs but is clearly visible in radioautographs prepared from normal cells. The radioautographs are otherwise identical. In some cases, polyoma-transformed cells for instance, there is not a complete loss of the high (greater than 200,000) molecular weight band, but in all cases a substantial reduction is seen in the amount of iodinated material in this region of the gel. Hynes further shows that the band in normal cells is particularly sensitive to proteolysis of intact cells and can be removed from the surface of normal iodinated cells significantly faster than the other bands that were progressively degraded during more extensive proteolysis.

The same experiments were repeated with a representative collection of normal cell lines (hamster, mouse, chick) and their viral transformants (hamster sarcoma, polyoma, RSV) with essentially the same results. Furthermore, the differences observed could not be related to the state of growth of the cells since confluent quiescent cells and non-confluent rapidly dividing cells gave identical iodination patterns.

The conclusions from these experiments clearly mean that a protein, apparently a glycoprotein, is available to lactoperoxidase in many normal cell lines but is not iodinated after transformation. Either the glycoprotein is missing entirely from the cell surface of transformed cells and presumably is not synthesised by these cells, or the glycoprotein is present on the surface membrane but is not accessible for iodination. It will be recalled that this second explanation was originally used for the decreased agglutination of normal cells by concanavalin A or wheat germ agglutinin. Subsequent attempts to demonstrate differences in binding of labelled lectins by normal and transformed cells have, however, in general been unconvincing. In the best case an increase of only 3-5 fold in transformed cells is claimed (Noonan and Burger, *J. biol. Chem.*, **248**, 4286; 1973). Clearly the increased labelling of the high molecular weight band by lactoperoxidase treatment of normal cells compared with transformed cells is considerably more impressive than these values.

There is the intriguing possibility that the iodinateable band is present transiently in transformed cell membranes but is released by a high level of endogenous protease. The amount of proteolytic digestion that removes the band from iodinated normal cells is about the same as that which causes normal cells to grow to high cell densities, that is to escape temporarily growth control and act as transformed cells. Whether or not these events are mere coincidences remains to be established. It is tempting, however, to infer a causal relationship between a high level of endogenous proteolytic activity in transformed cells, loss of the high molecular weight surface glycoprotein and insensitivity to contact inhibition of growth. It will now be of great interest to characterise further the glycoprotein and to establish what function if any it has in the events of contact inhibition and growth control. Its ready release from cells by mild proteolysis may very well contribute to this end.

Sediments in archaeology

from a Correspondent

An interdisciplinary discussion on sedimentological analysis in archaeology was organised by D. A. Davidson (St David's University College, Lampeter) and M. L. Shackley (University of Southampton), at the University of Southampton on December 15–16. Participants were drawn from a wide range of disciplines—archaeology, pedology, geography, geomorphology, geology and the biological sciences. The meeting was financially supported by the British Academy and the Royal Society.

Attention was first directed towards methods of field recording of archaeological sediments and their subsequent laboratory analysis. Controversy became apparent over the longterm usefulness of sediment recording systems. On the one hand there is need to record as much information as possible on an archaeological site but on the other there is always the danger that the recorded data may become obsolete with the development of new problems and techniques. J. Catt and A. Weir (Rothamsted Experimental Station), in a review of laboratory techniques, stressed the need for selectivity in sample size and analytical technique because most methods are very time consuming. The use of petrographic, heavy mineral and arc spectrographic techniques in assessing the provenance of sediments used in ceramics was demonstrated by J. LL. W. Williams and D. A. Jenkins (University College of North Wales, Bangor). Consideration of specific techniques was followed by discussion of coastal palaeoenvironments and the chronology of sea level change. C. Delano-Smith (University of Nottingham), on behalf of I. Morrison (University of Edinburgh), discussed an analysis of more than 1,000 radiocarbon-dated strata directly involved in marine changes along the western seaboard of Europe during the Holocene. It was suggested that eustatic change during this period has been marked by short term oscillations rather than by gradual changes.

Much of the meeting was devoted to terrestrial sedimentary environments with specific reference to human occupation. A. V. T. Kirkby and M. J. Kirkby (University of Leeds) have established an association between form and age of house mounds at sites in the Oaxaca Valley, Mexico. Consideration of site burial by alluviation also suggests that surface evidence by a scatter of sherds is quickly lost. A. Straw (University of Exeter) by describing a site at Welton-le-Wold in Lincolnshire presented a neat example of the use of geomorphological and sedimentological techniques in the reconstruction of a Pleistocene environment. A similar approach was adopted by A. J. Tankard and F. R. Schweitzer (University of Cape Town) in their analysis of the intricate cave sediments at die Kelders, Cape Province; parameters of grain size provide clues to former sedimentary environments within the cave. The relations between distribution of sites and spatial variation in sediment was also considered; for example, J. L. Bintliff (University of Cambridge) examined pre-Roman settlement patterns in selected parts of southern Greece and illustrated a spatial relation between sites and fertile soils. A sedimentological approach to settlement patterns was also adopted by C. Delano-Smith for the Tavoliere of Foggia, Italy.

The final part of the meeting was devoted to the biological characteristics of sediments. The results of pollen analysis have been widely utilised in archaeology and G. W. Dimbleby (Institute of Archaeology, London) summarised problems in the interpretation of palynological evidence. Contributions by P. Buckland (Doncaster) on insect remains and J. Evans (University College, Cardiff) on molluscan faunas concluded the meeting. The proceedings will be published in 1975 by Duckworth.

Control of Cell Division by Very Lysine Rich Histone (F1) Phosphorylation

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F1 histone phosphorylating activity correlates closely in the cell cycle with the behaviour of the mitotic trigger. It is proposed here that the phosphorylation of F1 histone is the initiation step for mitosis.

THE control of mitotic cell division, a universal and fundamental aspect of all eukaryote life, is the basis of the cancer problem. The control process involves an interaction between the nucleus and the cytoplasm and here we present a proposal for two steps, at the molecular level, in this interaction leading to mitosis. The proposal involves phosphorylation of F1 histone, which is controlled, presumably from the cytoplasm, by a nuclear F1 histone phosphorylating activity. We present here detailed experimental data in support of the proposal and suggest methods for further testing.

Histones have been implicated in most chromosome functions by various authors although it now seems unlikely that functions, such as specific repression, which require a wide range of tissue and species specificity can be carried out by histones. Indeed, the remarkable conservation of the F2A1 histone primary structure during evolution[1] argues strongly that this histone, at least, performs a function that is exactly the same in all eukaryotes and that involves a very precise molecular interaction. One probable function of the four histones F2A1, F2A2, F2B and F3 is the maintenance of the normal interphase tertiary structure of DNA. It is clear that the aspects of this structure observed in calf thymus chromatin by X-ray diffraction are not caused by F1 histones[2].

Post-synthetic covalent modifications of histones have been observed and may be important in the function(s) of these histones. In one case, the phosphorylation of F1 histone, the modification is reversible and occurs in a cyclic fashion[3,4] with both old and newly synthesised histone being phosphorylated[5]. We have shown that the maximum phosphate content of the very lysine rich histone in *Physarum polycephalum* (Pp F1) occurs in late G2 phase at exactly the time that chromosome condensation before mitosis becomes visible in the phase contrast microscope (±0.005 of a generation time). Data from mammalian systems are fully consistent with these observations. This and other experiments led to our proposal that phosphorylation of lysine rich histone causes chromosome condensation[3].

To study the control of Pp F1 phosphorylation, chromosome condensation and mitosis, it is important to know if the phosphorylation is caused by a change in enzyme activity or by a change in substrate availability. Substrate availability could be effected by a change in chromatin structure and, if this were found to be the controlling factor, Pp F1 phosphorylation might be a trivial consequence of chromosome condensation. On the other hand, a change in the enzyme activity would strongly imply that Pp F1 phosphorylation is a necessary preliminary step in chromosome condensation. Changes in histone phosphate content can be achieved by the action of a histone phosphokinase or a histone phosphatase[6]. The factor controlling histone phosphate content is the net histone phosphorylating activity in the nucleus. Here we report the results of a series of measurements of the net phosphorylating activity of nuclei of *P. polycephalum*, isolated at defined stages of the mitotic cycle, acting on added calf thymus F1 histone.

P. polycephalum is a true slime mould. In the plasmodial stage the nuclei go through mitosis synchronously and such synchronous divisions can be maintained for at least five mitoses. The nuclei behave like typical eukaryote nuclei except that the nuclear membrane remains intact during most, if not all, of mitosis. The mitotic cycle time of our specimens is 8 to 9 h; G1 phase, if it exists at all, is less than 10 min; S phase is about 3 h and G2 phase is about 5 h. *P. polycephalum* histones resemble calf thymus histones[8,7]. It is assumed here that Pp F1 has the same function and mode of action as calf F1 histone, as the amino acid compositions are very similar, although it is known that Pp F1 moves slower during polyacrylamide gel electrophoresis[8,7].

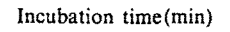

Fig. 1 Histone phosphorylating activity in *P. polycephalum* nuclei. Microplasmodia of *P. polycephalum* were grown in shake flasks in a semi-defined medium[42]. Nuclei were isolated by a modification of the method of Mohberg and Rusch[43] in which $MgCl_2$ was substituted for $CaCl_2$, the sucrose concentration was increased from 1.0 M to 1.6 M and a Polytron sonicator was used instead of a Waring blender. The nuclear pellet was washed twice with 10 mM $MgCl_2$+10 mM Tris-HCl, *p*H 7.2. Each incubation mixture contained 50 μmol Tris-HCl, *p*H 7.2, +5 μmol $MgCl_2$+1.5 nmol cyclic 3′,5′-AMP+0.5 μmol theophylline+1 mg calf F1 histone+10^8 nuclei in a final volume of 1.5 ml. The reaction was started by the addition of 1 μmol γ-^{32}P-ATP (1 to 2 Ci mmol^{-1}). After incubation at 25° C the reaction was stopped by the addition of an equal volume of 10% trichloracetic acid (TCA) containing 5 mM sodium pyrophosphate and 1 mM sodium phosphate[10]. After centrifugation of the assay mixture at 2,000*g* for 20 min, the pellet was extracted with 1 ml 0.2 M $CaCl_2$ at 80° C for 5 min. The mixture was centrifuged as before and the two supernatants pooled. 1 mg calf F1 histone was added as carrier and TCA was added to a final concentration of 25%. The histone was purified by four washes in 25% TCA and extraction of the precipitate with 0.02 N H_2SO_4 by dialysis overnight at 4° C. The extract was centrifuged at 20,000*g* for 30 min and the supernatant radioactivity determined by Čerenkov counting in a liquid scintillation counter[44].

Nuclear histone phosphorylating activity

P. polycephalum nuclei were isolated, sonicated and incubated in the presence of γ-^{32}P-ATP, calf F1 and cylic 3′5′-AMP as described in the figure captions. ^{32}P incorporation into calf F1 was demonstrated by acrylamide gel electrophoresis of the radioactive product. All the radioactivity migrated with the calf F1 band, located by staining (R. J. I., PhD thesis, in preparation). The time course of incorporation was somewhat unusual. With the lower ratio ATP: nucleus the incorporation occurred in the first 2 min followed by a slower net removal of ^{32}P from the substrate (Fig. 1). These kinetics are tentatively interpreted as being due to the combined action of a phosphokinase activity and a phosphatase activity[6]. Initially, the ^{32}P ATP concentration is high and the ^{32}P calf F1 specific activity is zero, so only the phosphokinase reaction is observed. Later, however, the ATP concentration drops and the ^{32}P F1 activity rises so that the phosphatase reaction becomes progressively more important and eventually dominates. The kinetics can be predicted qualitatively by a simple mathematical model based on these assumptions (H. R. M., unpublished). As would be expected, increasing the initial ^{32}P-ATP: nucleus ratio shifts the position of the maximum incorporation to longer times (Fig. 2).

P. polycephalum nuclei were isolated at defined stages of the naturally synchronous mitotic cycle and their net phosphorylating activity measured. Figure 2 shows the results obtained with nuclei isolated in S phase or in G2 phase. There is clearly a large increase in net phosphorylating activity in G2 phase. Figure 3*b* summarises the results obtained with nuclei isolated from many points in the mitotic cycle. The F1 phosphorylating activity increases steadily through the mitotic cycle from a minimum near metaphase to a maximum in late G2 phase, 1.75 h before metaphase, and then falls very rapidly to the minimum near metaphase. This pattern is quite different from that observed for most *P. polycephalum* enzymes[9]. The increase in phosphorylating activity precedes the increase in Pp F1 phosphate content as would be expected if the change in phosphate content were caused by the change in phosphorylating activity. Figure 3*c* shows the startling result of plotting the logarithm of the net F1 phosphorylating activity as a function of time in the cycle. It is clear that the increase in activity per nucleus follows a pure exponential law from early S phase to late G2 phase. The extremely rapid decrease in activity just before metaphase also follows an exponential law but the time period is so short that other interpretations may be possible.

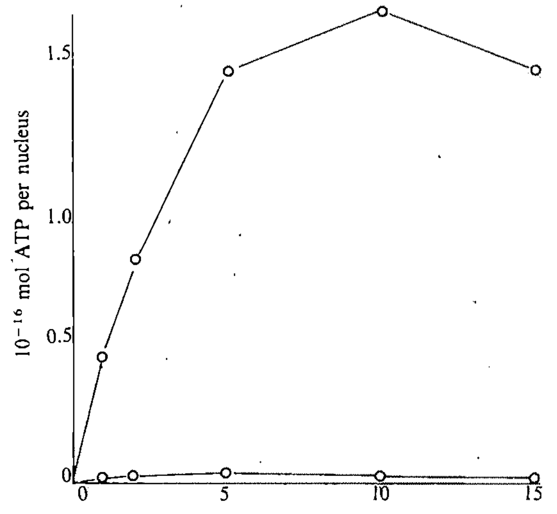

Fig. 2 Histone phosphorylating activity in G2 and S phases. Synchronous plasmodia of *P. polycephalum* were cultured on filter paper[45] and the stages of the mitotic cycle (M) were determined by microscopic examination of glycerol : ethanol fixed smears under phase contrast microscopy[42]. The assay was performed as described in the caption to Fig. 1 except that the number of nuclei was reduced to 1.7×10^7 per 1.5 ml and the first 25% TCA precipitate was washed and counted as described by Lake and Salzman[10]. Using the known specific activity of the ^{32}P-ATP and the known concentration of nuclei (counted in a haemocytometer) the results were expressed as mol phosphate transferred to F1 per nucleus. The F1 was present in excess (R. J. I., PhD thesis, in preparation). ●, Obtained using nuclei isolated 1 h after metaphase, that is in mid S phase; ○, obtained from nuclei isolated 1 h before metaphase, that is in late G2 phase.

Table 1 Comparison with Mitotic Stimulation Data

Stage of donor plasmodium h before mitosis	Fraction of generation time	Acceleration of mitosis[37] (h)	Phosphorylating activity mol ATP per nucleus $\times 10^{-21}$
7.00	0.82	0.42	30
4.50	0.56	0.42	100
3.00	0.35	1.08	210
1.25	0.15	0.92	110
0.33	0.04	0.67	50

F1 phosphate content

The temporal correlation between Pp F1 phosphate content and chromosome condensation before mitosis indicates that phosphorylation induces the necessary preliminary structural changes in chromatin[3]. This is supported by the observations, in mammalian systems, of high F1 phosphate content in metaphase chromosomes[10] and in late G2 and mitotic cells from synchronised cultures[11], and by the very widespread correlation between F1 phosphate content and growth rate[12]. The data on Pp F1 (Fig. 3*a*) show no increase in overall phosphate content during S phase. If, however, the data are recalculated to simulate the type of pulse labelling experiment that has been carried out in mammalian systems[13], then a second peak of phosphorylation appears in S phase as well as the first peak in late G2 phase (H. R. M., unpublished). The second peak is due to the fact that histones are synthesised during S phase and some phosphorylation of new histone is required to maintain the low 'background' F1 phosphate content. There is no evidence that this phosphorylation is connected with DNA synthesis, although a connection is not ruled out.

Several *in vitro* studies have cast histone F1 in the role of a cross linking agent in chromatin[14–18]. Moreover, a reversible *in vitro* condensation of chromatin can be induced by salt changes and this process does not occur with F1 depleted chromatin[19]. Nuclear magnetic resonance studies of the salt-induced condensation have suggested that the lysines of the basic ends of F1 always remain bound to the remaining DNA : protein complex but that the central 'interacting segment' has at least two conformations, one producing dispersed chromatin and the other(s) producing condensed chromatin[20]. It is interesting that the phosphate sites in *in vitro* phosphorylated F1 histone are situated in the basic ends of F1 and not in the 'interacting segment'[6,21]. A model of chromatin condensation based on phosphorylation is presented in Fig. 4. It is consistent with the observed weaker binding of F1 to DNA in condensed chromosomes[22]. As Pp F1 phosphate content falls during mitosis it is proposed that F1 phosphorylation is the initial mechanism of condensation and that other mechanisms are involved later, possibly disulphide bridges between F3 histones[23]. This proposal is consistent with the fact that metaphase chromosomes are stable while phosphokinase activity decays during metaphase arrest[10,24].

It has been proposed recently that F1 phosphorylation controls interphase chromosomal coiling[4]. It is difficult, however, to reconcile such a proposal with the lack of effect of F1 on this structure *in vitro*. Further, the model is based on an early result that euchromatin F1 is highly phosphorylated[25], while the more recent substantial criticisms of the methods by which this result was obtained[26-28] have not been discussed[4].

Initiation of mitosis

P. polycephalum is an excellent model system for the study of the control processes involved in the initiation of mitosis and therefore mitotic cell division. A considerable weight of data has been accumulated describing the properties of a supposed 'mitotic trigger' substance or substances[29] but no serious suggestions have yet been made concerning the molecular nature of the 'trigger' except that it may be protein[30]. Its predicted properties, however, are very close to the observed properties of the F1 phosphorylating activity in *P. polycephalum* nuclei as reported here. We propose, as a working hypothesis to be tested, that F1 phosphorylation is the initiation step for mitosis and that this step is 'triggered' or controlled by net F1 phosphorylating enzyme activity.

The 'mitotic trigger' has been observed directly by adding a homogenate of a plasmodium relatively near to mitosis to a growing plasmodium relatively far from mitosis. Significant stimulation was observed and the nearer the homogenised plasmodium was to mitosis the greater its stimulatory effect until a maximum was observed[30]. These experiments have been criticised on the grounds that they were found to be difficult to reproduce and the possibility of the effect being due to a general growth factor has not been ruled out (J. Mohberg, unpublished).

The results are in general agreement with the more widely accepted fusion experiments and we are inclined to accept them in the absence of any well documented alternative observation. Our proposal predicts a stimulatory effect from plasmodia at all stages of the mitotic cycle with a maximum 1.75 h (0.2 of a generation time) before mitosis, in agreement with Oppenheim and Katzir[30]. Table 1 shows the agreement between the observed acceleration of mitosis[30] and the net F1 phosphorylating activity of nuclei (interpolated from the data of Fig. 3). The results are consistent, within the probable experimental error, with the conclusion that the stimulating effect is proportional to the F1 phosphorylating activity, in strong support of our proposal.

A slightly less direct but more popular approach to this problem has been to use the fusion between plasmodia at different stages of the mitotic cycle. The reaction involves fusion of the cytoplasm[31] and diffusion of nuclei from one cytoplasm to another[32]. In all fused plasmodia the nuclei all divide synchronously at a time intermediate between the division times in the homogenous controls[33-36] unless one of the donor plasmodia is close to division, in which case nuclei from this plasmodium are not affected by the fusion[32,37]. This 'point of no return' occurred less than 0.14 of a generation time before metaphase. Our model does not lead to an accurate prediction of the earliest 'point of no return' but it would be expected to occur not later than the time when the phosphate content of Pp F1 had risen well above the early G2 phase level. At 0.14 of a generation time before metaphase the phosphate content was three to four times the early G2 phase level, two thirds of the total change having already occurred. This is clearly consistent with the observed earliest 'point of on return' which is thus probably associated with the phosphate content of Pp F1.

The stimulation or delay of mitosis observed with plasmodia fused at other times is probably associated with the cytoplasmic synthesis of F1 phosphokinase or its activator or repressor. The fusion experiments strongly implicate the cytoplasm[33] and ultraviolet irradiation experiments have been interpreted in the same way[37]. Our model thus predicts that the stimulation of mitosis should increase with the time difference between the fused plasmodia, in agreement with Chin *et al.*[32]. In fact, if the stage of the fused plasmodium corresponds to the stage that would have the same mean level of F1 phosphorylating activity as the fused plasmodium has immediately after fusion, then the stimulation can be predicted directly (Fig. 3*c*), with no arbitrary

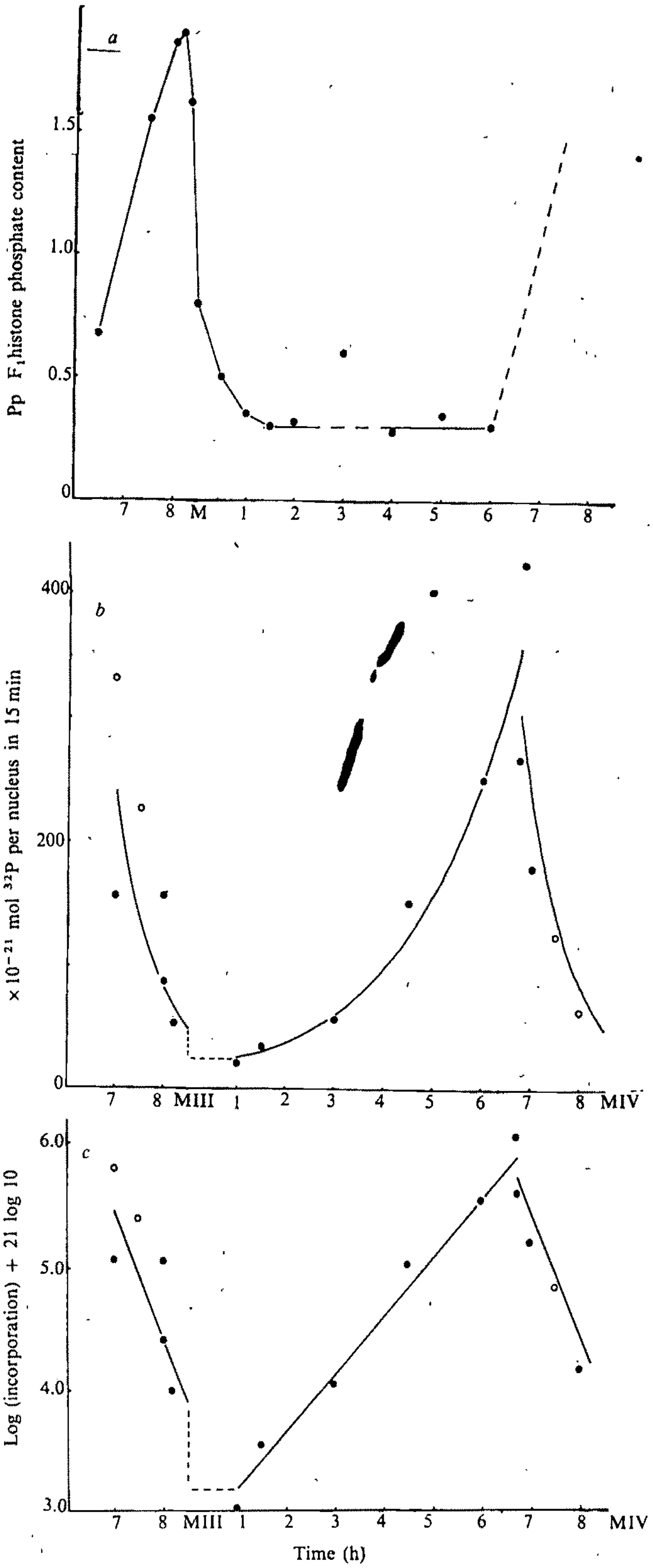

Fig. 3 Variations during the mitotic cycle. *a*, Phosphate content of Pp F1 on an arbitrary scale[3]; *b*, net F1 phosphorylating activity of *P. polycephalum* nuclei determined as described in the caption to Fig. 2. ●, Results obtained with 1.7×10^7 nuclei per 1.5 ml; ○, obtained with different concentrations of nuclei. The incorporation was assumed to be proportional to the concentration of nuclei. ——, The equation; incorporation$=15 \times 10^{-21}$ $\exp(0.47t)$, $1<t<6.75$ (Equation 1); incorporation$=340 \times 10^{-18}$ $\exp(-1.04t)$ $6.75<t<8.3$; where t is time in h after the previous metaphase, - - - -, Extrapolation obtained assuming the incorporation per nucleus falls by 50% at division. *c*, The data of *b* plotted on a logarithm scale. The straight lines, equations above, were fitted by least squares. The regression coefficients are $r=0.99$, $1<t<6.75$; and $r=0.79$, $6.75<t<8.5$.

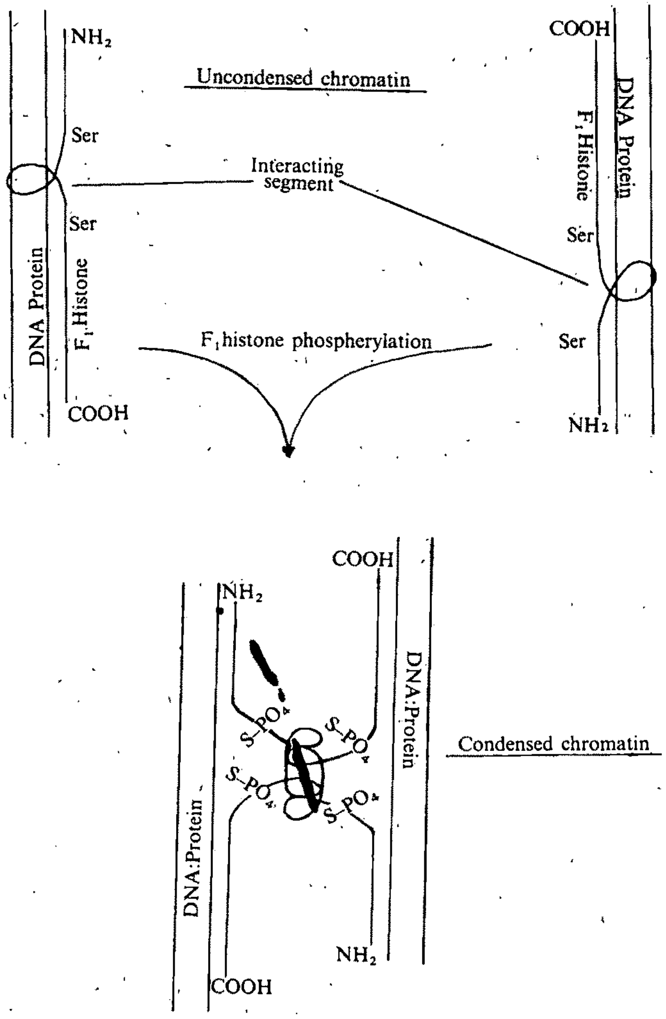

Fig. 4 Condensation of chromatin before metaphase. S-PO$_4$ and ser are the phosphorylation sites on F1; ser represents the nonphosphorylated form and S-PO$_4$ represents the phosphorylated form. The diagram is not intended to specify the precise details of the interactions, although a convincing series of inter- and/or intra-molecular attractions and repulsions are present[20]. In interphase (uncondensed) chromatin it is proposed that the 'interacting segment' of F1 is loosely bound to DNA: protein. Phosphorylation of the serines at the ends of this segment would tend, due to repulsion from DNA phosphates, to lift the interacting segment away from DNA: protein. Such a tendency would increase the probability of an intermolecular interaction between F1 histones as shown above and such interactions would bring about the observed chromosome condensation. The data at present available do not rule out similar models involving only intramolecular conformational changes, nor is it essential that the phosphorylation sites be exactly where shown.

Table 2 Comparison with Plasmodial Fusion Data

Unfused control time to mitosis*	Unfused time difference between plasmodia	Observed stimulation of mitosis[36]	Calculated stimulation of mitosis
		±0.04	
0.49	0.34	0.21	0.22
0.51	0.30	0.22	0.19
0.67	0.40	0.26	0.27
0.58	0.31	0.20	0.20
0.42	0.13	0.09	0.07
0.38	0.06	0.05	0.03
0.47	0.17	0.10	0.10
0.56	0.18	0.10	0.11
0.69	0.29	0.16	0.18
0.52	0.10	0.09	0.06
0.62	0.18	0.13	0.11
0.69	0.25	0.13	0.16

*All times are fractions of one generation time.

constants being required. The calculation of predicted mitosis stimulation for the times investigated by Chin *et al.*[32] shows (Table 2) that the calculated value is always the same as the observed value within the experimental error; clear support of our proposal. The strength of the support is limited by the accuracy of the data. The effect of elevated temperature shocks on the timing of mitosis in *P. polycephalum*[38] is also in agreement with our proposal. *In vitro* pre-incubation for 30 min at 37° C reduces the F1 phosphorylating activity to about 50% of normal (R. J. I., unpublished). Thus our proposal predicts that the effects of temperature shock should be minimal in early S phase, rising exponentially to a maximum about 1.75 h before metaphase and then falling sharply. This is exactly the pattern observed by Brewer and Rusch[38]. They also observe a transition point at 16 min before metaphase where temperature shock prevents mitosis altogether instead of just delaying it. This corresponds in time to the onset of the proposed second stage of chromosome condensation.

The proposal does not make any particular predictions about the levels of protein and nucleic acid synthesis required for the initiation of mitosis. It is worth noting, however, that the inhibition of protein synthesis does not prevent the completion of mitosis if the inhibitor is added in late prophase[39,40] just after the peak of Pp F1 phosphate content[3], thus supporting our proposal. The initiation of mitosis seems to be dependent on previous DNA synthesis[37] but not actinomycin D-sensitive RNA synthesis[41].

We conclude that phosphorylation of F1 initiates chromosome condensation which is then completed by a second process; that this phosphorylation is controlled by net F1 phosphorylating enzyme activity; and that these processes represent the two main steps in the initiation and control of initiation of mitotic cell division. These are consistent with the experimental data available, but to test these proposals further an attempt is being made to stimulate mitosis by adding purified phosphokinase to growing plasmodia of *P. polycephalum*. Other tests would be to study F1 phosphokinase activity in fused, temperature shocked, drugged or irradiated plasmodia. If the proposals are correct it may be possible to inhibit mitotic cell division without affecting other normal cell functions using F1 histone phosphatase. This would apply to cancer as well as normal cells[12].

Finally, it should be pointed out that although the evidence for these proposals is widespread, it is all, so far, circumstantial and more direct experiments are needed to confirm or deny them.

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Evolution in Action

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The evolution of enzymes in bacteria is studied by introducing novel substrates and selecting strains that have mutated to be able to use these substrates.

THE present day bacteria possess enzymes which are the end products of millions of years of evolution, but there is no reason to suppose that their evolutionary potential is at an end. Bacteria that live in soil and water encounter a vast array of organic compounds, arising from natural biosynthetic processes, which can be utilised as growth substrates. These have been augmented in recent years by the products of the chemical industry, thereby creating selection pressures for the evolution of enzymes for the degradation of man-made molecules. Catabolic pathways are known for such compounds as camphor, naphthalene, phenol, catechol, cresols, alkanes and also for synthetic detergents such as benzene sulphonate, and herbicides, for example, 2,4-dichlorophenoxyacetate (2,4-D). For many of these catabolic pathways the detailed enzyme reactions have been established and in some cases the genetic determinants have been analysed[1]. The ability to metabolise organic compounds depends on a number of factors including the possession of enzymes with the appropriate specificities, transport systems to get the potential growth substrate into the cell and regulatory mechanisms that can be activated when required.

We have taken a single enzyme, an inducible aliphatic amidase of *Pseudomonas aeruginosa*, and shown that it is possible to evolve amidases with altered substrate specificities which enable mutants to utilise amides that do not support growth of the wild-type parent[2–4] (Table 1). These changes in enzyme specificity involve very few changes in the primary structure of the enzyme protein and arise from a small number of single-site mutations. A comparison of a number of independent isolates of *P. aeruginosa* showed that they were able to utilise the same amides for growth; the substrate specificities of their amidases, and the apparent K_m values obtained for the various amides, were the same within the limits of the measurements made. Thus, the substrate specificity of the aliphatic amidases of wild-type strains of *P. aeruginosa* seems to be a stable character for this species, although it can be changed in the laboratory under the appropriate selection pressure.

The first systematic studies of the evolution of strains with the ability to utilise novel growth substrates, were made by

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Wood and Mortlock and their colleagues who investigated the growth of various bacterial species on pentoses and pentitols[6]. They found that the most common mutation which allowed the use of a novel growth substrate was a mutation conferring constitutivity on an enzyme that already had some affinity for the compound. The system was thus freed from the constraints of inducer specificity; since constitutive mutants can produce large amounts of enzymes (2% or more of the total cell protein), the amount of the enzyme compensated for its feeble activtiy. In one case a regulatory mutation produced a mutant with altered inducer specificity that could be induced by the novel growth substrate[7]. This is a more satisfactory biological solution since derepressed strains are at a growth disadvantage compared with wild-type strains under growth conditions which do not require the derepressed enzyme[8]. This is because they expend energy on synthesising the constitutive enzymes even when they are of no metabolic value to the cell. Mutations in the structural genes to produce enzymes with increased affinities for their novel substrates, have been described for L-fucose isomerase[9] and ribitol dehydrogenase[10]. The evolutionary sequence established by Wu, Lin and Tanaka[10] for an increased rate of growth of *Klebsiella aerogenes* with xylitol was first, a mutation resulting in a constitutive ribitol dehydrogenase, second, a mutation increasing the affinity of ribitol dehydrogenase for xylitol, and third, a mutation which allowed xylitol to be transported more rapidly into the cell. In a detailed investigation (unpublished) of selection in continuous culture, B. S. Hartley, P. W. J. Rigby, and B. D. Burleigh, jun., found that mutations leading to gene amplification also increased the rate of growth of this organism with xylitol.

Table 1 *Pseudomonas aeruginosa* amidase mutants shown in Figs 1 and 2

Strain No.	Series No.	Phenotype	Genotype
PAC1	Wild	Ind A^+B^-	*amiR⁺amiE⁺*
PAC111	C11	Con A^+B^-	*amiR11amiE⁺*
PAC153	F6	F-ind A^+B^-	*amiR43amiE⁺*
PAC142	L10	Con A^+B^+But-r Crp-r	*amiR33amiE⁺crp-7*
PAC128	CB4	Con A^+B^+But-r	*amiR11,37amiE⁺*
PAC351	B6	Con A^+B^+	*amiR11amiE16*
PAC366	AI3	Con $A^+B^-AI^+$Crp-r	*amiR33amiE56crp-7*
PAC326	LAml	(Ind)A^-(Crp-r)	*amiR33amiE34crp-7*
PAC392	PhF1	ConA(+)$B^+V^+Ph^+$	*amiR11,37amiE82*
PAC377	PhB3	Con $A^-B^+V^+Ph^+$	*amiR11amiE16,67*
PAC353	V2	Con $A^-B^+V^+$	*amiR11amiE16,23*
PAC356	V5	Con $A^-B^+V^+$	*amiR11amiE16,26*
PAC360	V9	Con $A^+B^+V^+$	*amiR11amiE16,30*
PAC391	PhA1	Con $A^{(+)}B^+V^+Ph^+$	*amiR33amiE34,81crp-7*
PAC388	PhV1	Con $A^-B^+V^+Ph^+$	*amiR11amiE16,30,78*
PAC389	PhV2	Con $A^{(+)}B^+V^+Ph^+$	*amiR11amiE16,30,79*
PAC398	IB10	Ind A^+B^-	*amiR⁺amiE16*
PAC308	Am8	(Ind) A^-B^-	*amiR⁺amiE8*

The genotype is given as *amiR*, amidase regulator gene; *amiE*, amidase structural gene; *crp*, gene(s) concerned with catabolite repression. The phenotype is given for growth; +, good growth; (+), trace growth; −, no growth on minimal agar plates containing as carbon sources A, acetamide; B, butyramide; V, valeramide; AI, acetanilide and as nitrogen source only, Ph, phenylacetamide (refs 2–4, 13, 14 and J. E. Brown, J. L. B., P. H. C., and M. D., in preparation).

The aliphatic amidase of *P. aeruginosa* has a well defined specificity for amide hydrolysis and also a well defined inducer specificity (Table 2). The two amides that can be utilised for growth by the wild-type strain are acetamide and propionamide and the corresponding acids can be readily metabolised. *P. aeruginosa* grows well in simple salt media and can be grown on a scale which provides sufficient of the enzymes for protein studies. The amidase has a molecular weight of about 200,000 and is made up of six identical subunits.[11]

The successful isolation of mutants producing amidases with altered substrate specificities depended on exploiting the regulatory characteristics of the enzyme, and using a variety of different amides in the selection media. Amides can be used as nitrogen sources, carbon sources or both at the same time. The altered amidase mutants shown in Table 1 were all selected from plates in which the following amides provided part or all of the growth requirements: butyramide, valeramide, phenylacetamide and N-phenylacetamide. No mutants producing altered enzymes could be isolated directly from the wild-type strain and the regulatory phenotype of the parental strains was critical in determining whether or not a particular class of altered enzyme mutant could be obtained by this simple positive selection method. The strategy adopted in this attempt at experimental evolution was based on a detailed study of the mechanism of regulation of amidase synthesis[12,13]. As we discuss in detail later, some altered enzyme mutants were isolated only from parental strains which were maximally derepressed for amidase synthesis, while others could be isolated only from a particular class of repressible mutants.

Table 2 Amides and inducers, enzyme substrates and growth substrates for wild type *Pseudonmoas aeruginosa* PAC1

Amides	Relative rates of hydrolysis	Relative rates of induction	Amide analogue repression	Availability as growth substrate
Formamide	10	7	+	−*
Acetamide	100	100		+
Propionamide	300	50		+
Butyramide	2	0	+	−
Valeramide	0	0	+	−
Phenylacetamide	0	0	+	−
Acetanilide	0	0	+	−

* Formamide can be used as a nitrogen source for growth but not as carbon source since C_1 compounds are not utilised by this strain.

It can be seen from Table 2 that butyramide cannot be used as a growth substrate by the wild-type strain although it is hydrolysed by amidase at about 2% of the acetamide rate. Butyramide is unable to induce amidase synthesis in *P. aeruginosa* and furthermore, acts as a competitive inhibitor of induction by both substrate and non-substrate amides. It is thought to act by combining with the regulator binding protein produced by the amidase regulator gene at the inducer-binding site, without producing the conformational change necessary to enable expression of the amidase structural gene. This imposes an additional constraint on the regulation of amidase synthesis. Other amides act in this way and we refer to them as amide analogue repressors[12].

Butyramide-utilising mutants were isolated spontaneously from the wild type on butyramide plates at a frequency of about 10^{-6} and all were constitutive, producing wild-type A amidase (CB group). This is another example of a mutation to constitutivity conferring the ability to utilise a poor substrate. But, not all constitutive mutants grow on butyramide. Earlier, we had isolated constitutive mutants from succinate+formamide (S/F) plates, with succinate providing the carbon source and formamide the nitrogen source (C group). Formamide is a poor inducer and a poor substrate for the amidase; the wild type grows very slowly on this medium and constitutive mutants are readily isolated. About half of the constitutive mutants obtained from S/F plates are butyramide-positive in phenotype, and about half are butyramide-negative. The constitutive mutants that were unable to grow on butyramide were found to have retained the sensitivity to amide analogue repression which had been exhibited by the wild-type strain[14].

The first of the mutants producing an altered enzyme was obtained by selection on butyramide plates after mutagenesis of strain C11, a constitutive mutant with a butyramide-negative phenotype[2]. This B group of mutants retained the regulatory phenotype of the parent strain, and amidase synthesis was repressed by amide analogues. All the B group mutants seemed to produce the same amidase and mutant B6 was used to isolate the new enzyme, B amidase (Tables 1 and 3). B amidase differs from the wild-type A amidase in electrophoretic mobility, but is thermostable and gives complete cross reaction with antiserum to A amidase. B amidase is only slightly less active towards acetamide than is A amidase, and has increased activities for both propionamide and butyramide. The K_m for butyramide is decreased almost ten-fold and the V_{max} is increased ten-fold; the relative specific activities of B amidase for acetamide and butyramide are 100:30 whereas for A amidase they are 100:2. Although butyramide is still capable of repressing amidase synthesis in the B group of mutants, the rate of hydrolysis of butyramide by the altered enzyme removes it very rapidly from the growth medium[14].

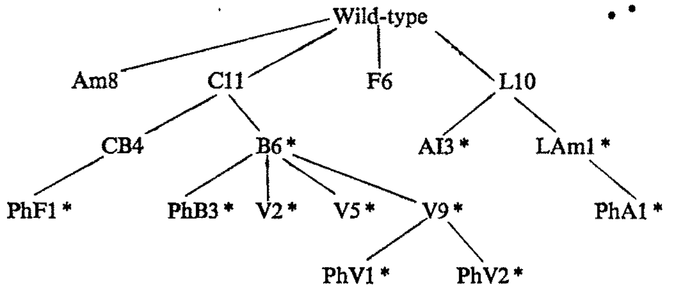

Fig. 1 Family tree of *Pseudomonas aeruginosa* amidase mutants derived from strain PAC1. Those producing mutant enzymes are marked with an asterisk, others are regulatory mutants or devoid of activity with all amides tested. Details of phenotypes and genotypes in Table 1.

A further mutational step in this evolutionary pathway produced the V class of mutants which are able to utilise valeramide for growth (Table 1 and Fig. 1). The V amidases are a heterogenous group and differ among themselves in substrate specificities and in thermal stability. The specific activities of all these enzymes were much lower than those of the wild-type A amidase or the mutant B amidase[2]. Within the bacterial cells they were stable, however, and this group of mutants grew quite well with valeramide both in liquid medium and on plates. One of the enzymes, V9 amidase, was cold labile but was successfully purified at room temperature. This class of mutant is expected to yield useful information on the amino acid substitutions affecting enzyme stability and the maintenance of catalytic activity.

The final class of mutants isolated by this route were two groups of phenylacetamide-utilising mutants, the PhB group and PhV1 and PhV2 (Table 1 and Fig. 1)[4]. All were derived from C11 through B6; the PhB group were derived in a single step from B6 while PhV1 and PhV2 were derived through V9. The amidases produced by these mutants are much less active than the wild-type enzyme, whether measured in whole cells or in extracts, but good growth occurs with phenylacetamide as the nitrogen source for growth. *P. aeruginosa* does not utilise the phenylacetate produced by hydrolysis of phenylacetamide so that an additional carbon source must be supplied.

A second route was also effective in obtaining mutants capable of utilising phenylacetamide. Strain CB4, one of the constitutive mutants with a butyramide-positive phenotype, was chosen as a potential parent strain; it was thought that its resistance to amide analogue repression might allow growth on phenylacetamide of mutants producing altered enzymes even if their specific activities towards phenylacetamide were not very high. Strain CB4 gave rise in a single mutational step to PhF1 (Fig. 1); the PhF1 amidase is one of the more unstable of the mutant amidases isolated so far.

Before mentioning the final evolutionary pathway which led to a phenylacetamidase, it is necessary to consider a further control which is imposed on amidase synthesis. Like many catabolic enzymes, amidase is subject to catabolite repression and one of the most potent of the repressing metabolites is succinate. In the presence of succinate as the carbon source and a low concentration of lactamide (a good inducer but a poor substrate) as nitrogen source, the wild type grows very slowly. This makes it possible to isolate mutants which are resistant to catabolite repression. Those that are both constitutive and resistant to catabolite repression, synthesise amidase to form about 10% of the total cell protein when grown in appropriate media. One of these mutants, L10, is used for routine preparation of A amidase, but is also useful as a parent strain for isolating mutant amidases. The large amounts of amidase protein produced by this strain means that the threshold of activity which allows growth on a substrate amide can be achieved, even with an enzyme of very low specific activity. A class of mutants producing defective amidases were isolated from strain L10 as parent. One of these, strain LAm1 (see Table 1 and Fig. 1), was the parent of the phenylacetamide-utilising mutant PhA1. The route by which PhA1 was derived, suggests that it has two separate mutational sites. Since PhA1 grows very slowly on acetamide it could, however, be regarded as a partial revertant and the mutation allowing growth on phenylacetamide might have occurred at the same site as the original mutation. The PhA1 amidase is very thermolabile and in this respect it resembles PhF1.

The most interesting of the mutant enzymes isolated so far is one that hydrolyses acetanilide (N-phenylacetamide) and allows growth with acetanilide as carbon source (Table 1). Mutant AI3 can also utilise acetanilide as nitrogen source, but growth is then slower since the hydrolysis product, aniline, is metabolised rather poorly. This mutant was derived directly from the catabolite repression-resistant mutant L10, which is also constitutive and resistant to amide analogue repression. The change in substrate specificity to allow the enzyme protein to accommodate the benzene ring, results in a decrease in the acetamidase activity, but this mutant is still capable of good growth on acetamide plates[3]. But while the parent strain L10 grows very well on butyramide plates, the mutant AI3 gives only trace growth since the specific butyramidase activity of the altered enzyme is very greatly reduced. The AI3 amidase can also attack *p*-substituted acetanilides, including *p*-nitroacetanilide, *p*-bromoacetanilide and *p*-chloroacetanilide (M. J. S. and P. R. B., in preparation). The A13 enzyme gives a complete cross reaction with antiserum to A amidase and is stable in

Table 3 Apparent K_m values ($\times 13^{-3}$ M) for wild type and mutant amidases

Substrate	Enzymes A	B	AI	PhB3	PhV1	PhF1
Acetamide	19	15	53	NP	NP	120
Propionamide	55	10	208	NP	NP	ND
Butyramide	500	74	ND	180	200	38
Phenylacetamide	NP	NP	NP	2	3	1

The enzyme was assayed by the acyl-group transfer reaction with hydroxylamine as acceptor (500 mM)[12]. ND, not determined; NP, not possible as no activity could be detected for that amide.

the cell-free state. It differs from both A and B amidases in electrophoretic mobility and the purified enzyme loses 40% of its activity on heating for 10 min at 60° C, while the A and B amidases are unaffected by this treatment. The difference between the AI3 amidase and the A amidase resides in the substitution of a single amino acid residue; a threonine residue of the wild-type enzyme is replaced by an isoleucine residue in the mutant AI3 enzyme[3].

Table 3 gives some of the apparent K_m values we have obtained for the wild-type and mutant amidases. The only case in which it was possible to compare K_m values for a novel growth substrate with both the parental and mutant enzyme was for butyramide with A and B amidases; the apparent K_m value for butyramide of A amidase was extremely high, 500 mM, while for B amidase it was 74 mM. Butyramide is one of the best substrates for the Ph group of enzymes and it can be seen that different K_m values for butyramide were obtained with the PhB3, PhV1 and PhF1 enzymes, which also differ from one another in other respects.

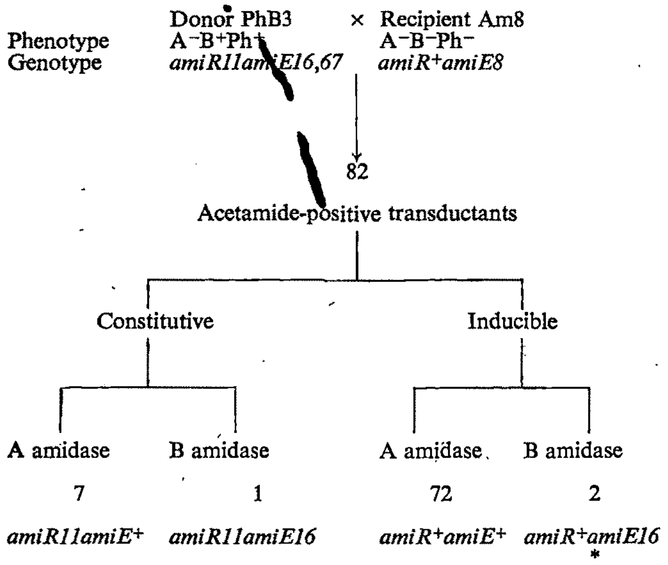

Fig. 2 Transductional cross between two amidase mutants which gave rise to some recombinants that produced the mutant B amidase and were inducible*. Other recombinant classes not shown above were presumed to be acetamide negative in phenotype.

No measurements with phenylacetamide could be made with amidases other than the Ph group since they had no detectable activity with this amide. It was found that the purified B amidase had trace activity for acetanilide and it is probable that this is also the case for the A amidase but no measurements have been made. The values given in Table 3 were all obtained using the acyl-group transferase reaction for assaying amidase activity[12].

The mutant amidases all have the same molecular weights and the same number of subunits; they all cross react with antiserum to the wild-type enzyme although some of the less stable mutant enzymes give rather diffuse bands. These less stable enzymes, particularly PhF1, PhV1 and some of the V amidases, should be of considerable value in looking at the subunit interactions of this protein. The existence of a series of very closely related enzymes also offers opportunities for exploring enzyme mechanisms by varying the enzyme structure rather than the substrate structure, and A, B and AI3 amidases are very suitable for this purpose.

Evolution of novel activities for *P. aeruginosa* amidase has been achieved experimentally by several different paths and it is not possible to say whether any of these routes might be followed in nature. There would be, for example, very little advantage for *P. aeruginosa* to have acquired a gene duplication and then to have evolved a phenylacetamidase. *P. aeruginosa* is unable to utilise phenylacetate, and shortage of carbon compounds, not nitrogen compounds, limits growth in the natural environment. We have, however, found that strains of *P. putida*, which are able to utilise phenylacetate, have evolved a phenylacetamidase which allows growth on plates with phenylacetamide providing both carbon and nitrogen source[15]. The *P. putida* phenylacetamidase is inducible by phenylacetamide as would be predicted. All our altered enzyme mutants are constitutive and are not yet suitable candidates for a natural ecosystem. They might be considered as strains in transition, for which the next step would require a mutation to allow them to be induced by the new substrate. We have constructed one inducible strain producing an altered enzyme, by recombination between PhB3 and an amidase-negative mutant (Fig. 3) (J. E. B., J. L. B., P. H. C., and M. Day, in preparation). The recombinant has a wild-type regulator phenotype and produces B amidase, but since it cannot be induced by butyramide this strain grows no better on butyramide than the wild-type. It does, however, open the possibility for new inducible strains to arise by recombination of various mutants.

Evolution by gene duplication is widely held to have played a major part in the evolution of enzymes[16]. Koch[17] suggests that to allow time and opportunity to acquire a sufficient number of mutations the duplicate gene should be retained unexpressed for a long period. We have shown for one enzyme at least, that very few mutations are needed to make considerable changes in substrate specificity which enable the organism to utilise new growth substrates. In *Pseudomonas* there is an additional genetic characteristic which may hasten the spread of an advantageous mutation through a population. Genes for several catabolic pathways have been found to be carried on transmissible plasmids; camphor[18], octane[19], salicylate[20] and naphthalene[21] plasmids have been identified. Genes for catabolic enzymes may be exchanged between chromosomes and plasmids, and new mutations offering a selective growth advantage may be spread much more rapidly through the microbial population than the classical interpretation of mutation and selection would predict.

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Sequence divergence of mammalian globin messenger RNA

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The rate of nucleotide change between globin genes for mouse, rabbit, rat and man is considerably slower than that of non-repeated DNA, suggesting that selection pressure is less stringent on the bulk of the genome than on genes coding for polypeptides.

THE role of the very large amount of DNA in the haploid mammalian genome is still obscure. There are simple sequences present at very high multiplicity which are not transcribed, of which the best studied is the satellite DNA of mouse. The bulk of the mammalian genome consists of a mixture of sequences many of which are present only in single copies, while others occur several hundred or thousand times. It is known that among the single-copy sequences are many of the genes coding for specific proteins, although some reduplicated sequences are also transcribed.

The function of the greater part of the mammalian genome is not clear. It has been suggested, from a consideration of the rate of accumulation of neutral mutations between species, that a large proportion of the genome may not code for protein[1,2]. A similar conclusion has been reached from a consideration of possible models for the control of gene expression[3-6]. A comparison of the rate of divergence of a single gene with the rate of divergence of the single-copy genome would indicate any difference in mutation rates between nucleotide sequences expressed as protein sequences and the total non-repeated DNA.

Sequence of globin gene

The nucleotide sequence of the globin gene is particularly suitable for a study of divergence, since it is known that the mRNA is complementary to, and thus synthesised from, sequences reannealing with the nonrepetitive fraction of the total DNA. These sequences are present as single, or at most two or three, copies[7-10]. Thus any changes in the nucleotide sequence of the gene will be reflected necessarily in the sequence of the globin mRNA. The single-copy DNA can be isolated using hydroxylapatite after allowing the rest of the DNA to reanneal, and its divergence between different mammalian species can be investigated and has been previously reported[11,12].

Globin mRNA can be isolated in pure form from reticulocytes of animal species[13-15]. As the amino acid sequences of globins from many different mammals are known, both the maximum and minimum divergence of the corresponding nucleotide sequences can be predicted, taking into account both amino acid differences between species and also possible third position changes in synonomous codons.

The rate of nucleotide change in the globin mRNA, and thus in the globin gene, has been determined by studying the extent of specific base sequence pairing (cDNA:RNA hybridisation) between a complementary DNA copy (cDNA) of the mRNA made with tumour virus reverse transcriptase and the mRNA of the same or different species. Nucleotide changes within the hybridised sequence can also be studied by determining the depression of the melting temperature of the hybrid which such mismatching causes.

We report here the degree of sequence divergence between the globin genes for mouse, rabbit, rat and man. In this way the actual rate of nucleotide change with evolution of this single functional gene has been found to be considerably slower than that of the total non-repeated DNA, although greater than the minimum which would be consistent with the differences in the amino acid sequence.

Globin mRNA was prepared from rabbit, rat and mouse reticulocytes by affinity chromatography to poly U-Sepharose[14-16]. In each case the mRNA gave a single major component at 9S on polyacrylamide gel electrophoresis. The human mRNA was prepared using similar techniques from circulating blood of an adult patient with chronic non-thalassemic anaemia. It was not possible to determine the electrophoretic purity of the small amount of human mRNA available.

Reverse transcriptase

Reverse transcriptase was prepared from avian myoblastosis virus through the CM-Sephadex purification step[17]. cDNA was prepared using purified mouse globin mRNA as template and oligo(T) as primer and was fractionated on an alkaline sucrose gradient to give a product of average molecular weight 120,000 (approximately 320 nucleotides)[7,18]. The cDNA was labelled with ^{3}H-dTTP and ^{3}H-dCTP (Radiochemical Centre, Amersham) and was at a final specific activity of 5×10^7 d.p.m. μg^{-1}.

The cDNA was hybridised in solution to a vast excess of mRNA. The hybridisation was analysed both by measuring resistance to S1 single strand specific nuclease[19] and binding to hydroxylapatite[20]. The stability of the hybrids was measured by the proportion eluted from hydroxylapatite with increasing temperature. Hybridisation of cDNA to mouse, rat and rabbit mRNA was for 18 h or less, to minimise the possibility of degradation of RNA affecting the rate; with human mRNA some hybridisations had to be continued for longer periods of up to 42 h because of the small amounts of RNA available. All points were determined in duplicate or triplicate.

TABLE 1 Homology between mouse cDNA and mouse, rat, rabbit and human globin mRNAs, as measured on hydroxylapatite.

Source of RNA	[mRNA] × $t_{\frac{1}{2}}$	% Reassociation	% Reassociation relative to mouse	T_m
Mouse	7.5×10^{-4}	80%	100%	76
Rat	2.3×10^{-3}	80%	100%	74
Rabbit	3.5×10^{-3}	50%	63%	69.5
Human	1.7×10^{-3}	30%	38%	69.5

Data are compiled from Figs 1 and 2.

The kinetics of annealing between mouse globin cDNA and mouse, rabbit, rat and human globin mRNAs as measured with hydroxylapatite are shown in Fig. 1. The half times and extent of reannealing are given in Table 1. Approximately 5% of the cDNA behaves as if double stranded even in the absence of driver RNA, and 20% fails to reanneal even for homologous hybridisation, as found previous for globin cDNA[7,18]. In principle if both cDNA and mRNA are intact and even a segment of the molecules anneals, one should measure 100% reassociation between mouse cDNA and rabbit or human mRNA when the hybrids are analysed on hydroxylapatite. The failure to do this indicates heterogeneity in the distribution of divergence in the population of molecules; either because they are smaller than the total message, or because a portion has accumulated more sequence divergence.

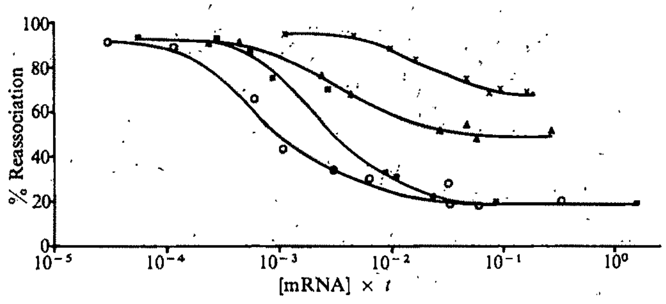

FIG. 1 Kinetics of reassociation of mouse globin cDNA with globin message from mouse, rat, rabbit and human. Reassociations were in 0.12 M phosphate buffer, pH 6.8, 0.06% SDS, at 60°C. Hybrids were analysed on 0.1 ml. hydroxylapatite columns at 60°C as described by Laird *et al.*[21] except that reassociated molecules were eluted with 0.3 M phosphate buffer, 0.06% SDS, 2000 c.p.m. of cDNA were used for each point. This was reacted to RNA which was in 10^4 molar excess over the cDNA. [mRNA] × *t* (mol s l^{-1}) is calculated from the initial concentration of RNA and the time of incubation[20]. ○—○, Mouse; ■—■, rat; ▲—▲, rabbit; ×—×, human.

The melting profiles obtained for hybrids formed after annealing was complete are shown in Fig. 2. The melting temperatures obtained are given in Table 1. Each melting profile was determined at least twice.

S1 nuclease sensitivity of the hybrids was also determined after annealing was complete (Table 2), under comparable conditions of stringency to those used in the hydroxylapatite experiments, as well as under conditions of lower stringency.

Sequence homology

The molecular weight of globin mRNA is approximately 220,000, of which 150,000 is coding sequence for the protein moiety. The cDNA copy used was approximately 60% of the length of the mRNA. The amino acid sequences for globin for the mammals studied differ by as much as 19% (Table 3). Since such changes can in most cases be generated by single base alterations, the minimum divergence predicted for nucleotide coding sequences is 7% for human and mouse (Table 3). But if all possible synonymous codon changes and maximum codon differences for amino acid substitutions are allowed a divergence of from 35% to greater than 40% in the coding region is predicted, and stable hybrids would not be formed under conditions employed for these experiments[24,25]. The sensitivity of the interspecific cDNA-mRNA hybrids to S1 nuclease demonstrates that more divergence has occurred in the mRNA sequence than the minimum predicted from the amino acid differences.

It is also possible to compare the divergence of globin mRNA sequences with that of the total non-repeated DNAs of the species studied. The melting temperature (T_m) of a DNA-RNA hybrid decreases with increasing base mis-pairing, and the difference between the T_m for a homologous and a heterologous hybrid (ΔT_m) is a measure of the divergence between the nucleic acids studied. Using comparable hybridisation conditions to ours McConaughy and McCarthy[11] found the homology between mouse and rat unique DNA to be 45% as compared with 100% for globin mRNA sequences, with a ΔT_m of 14°C as compared with 2°C. The homology between mouse and guinea pig is only 6% with a ΔT_m of 16°C; it is likely that rabbit and human non-repeated DNAs are even less like mouse. Kohne[12] has shown that under less stringent conditions 13% of human non-repeated DNA will react with rat DNA and that 70% of non-repeated mouse

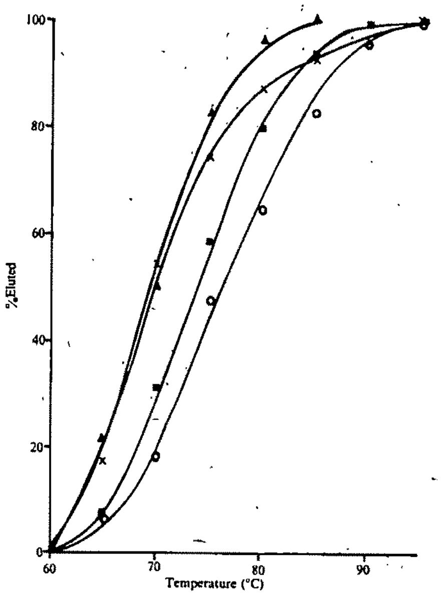

FIG. 2 Thermal dissociation of hybrids of mouse cDNA with globin mRNA from mouse, rat, rabbit and human. Hybrids were formed by reassociation to [mRNA] × *t* of greater than 10^{-1} as described in Fig. 1. The duplexes were bound to hydroxylatite and dissociated as described by Laird *et al.*[21]. The mouse, rat, and rabbit curves were determined simultaneously under identical conditions using 10,000 c.p.m. of cDNA per curve. It was necessary to determine the human T_m separately, using 5,000 c.p.m. of cDNA. ○—○, Mouse; ■—■, rat; ▲—▲, rabbit; ×—×, human.

DNA will hybridise with rat DNA with a ΔT_m of greater than 15°C. The homology between globin mRNAs is much greater, demonstrating that different degrees of selection operate on this coding sequence than on the genome as a whole. If this slow rate of divergence relative to total non-repeated DNA sequences is characteristic for other mRNAs, it may be concluded that much of the non-repeated DNA is not in sequences coding for proteins.

TABLE 2 Homology between mouse cDNA and mouse, rat, rabbit, and human globin mRNAs as measured with S1 nuclease.

	Homology relative to mouse Temperature of incubation	
Source of RNA	50° C	60° C
Mouse	100%	100%
Rat	97%	71%
Rabbit	79%	38%
Human	64%	32%
cDNA alone	15%	6%

Reassociations were performed in 0.18 M NaCl, 0.01 M Tris, *p*H 6.5 at the temperatures indicated to [mRNA] × *t* of greater than 10^{-1} in vast RNA excess. The reactions were cooled to 0°C and diluted to 0.18 M NaCl, 0.03 M sodium acetate bufer, *p*H 4.5, 10^{-5} M $ZnSO_4$, in the presence of 80 μg ml^{-1} of *Escherichia coli* RNA. S1 nuclease was added and the digestions were carried to completion by incubation at the temperature of hybridisation[19]. Nuclease-resistant hybrids were precipitated with TCA[22]. 800–1,000 c.p.m. cDNA were used for each point. Results are normalised to mouse for which 80% of the input was nuclease resistant.

cDNA used in these experiments was copied from purified mouse globin mRNA containing both α and β mRNA. The amino acid sequences of the mouse α and β globins are sufficiently different that the nucleotide sequences coding for them will not hybridise. The degree of amino acid divergence between species is similar for both chains (Table 3). It is assumed that the divergence seen between the nucleotide sequences similarly reflect changes in mRNAs for both chains. It is possible that a greater degree of nucleotide change has occurred in one chain than the other, as suggested by Housman *et al.*[26], but this is not apparent in the reassociation kinetics or melting profiles, which would be biphasic were divergence introduced into one of the two mRNAs preferentially. Leder *et al.*[24] have suggested from data similar to ours that the lack of homology between mouse and rabbit globin mRNAs may be accounted for by rapid divergence of non-coding sequences present in the gloin mRNA. Since the cDNA is transcribed from the 3′-end of the mRNA it is possible that as much as 43% of the transcript could be non-coding sequences, in the unlikely event that all of the extra nucleotides of globin mRNA are at the 3′-end of the molecule. But the close homology between mouse and rat globin mRNAs argues that there is little divergence in this case even for such cDNA sequences as may be derived from non-coding regions.

TABLE 3 Interspecific homology of mouse, rat, rabbit and human

		Predicted globin mRNA coding sequence difference	
Amino acid sequences of globin chains(23)	Amino acid % difference	Minimum	Maximum
Mouse α/rabbit α	19%	7%	40%
Mouse α/human α	13%	5%	35%
Mouse β/rabbit β	19%	7%	40%
Mouse β/human β	18%	7%	40%
Nonrepeated DNA			
	% cross-reaction	Δ T_m	
Mouse and rat[11]	45%	14° C	
Rat and human[12]	13%	Not determined	
Mouse and human or rabbit (calculated from ref. 11)	<6%	>15° C	

The data are equally consistent with a considerable amount of synonymous codon change having occurred in the message. For human globin cDNA, it has been reported that although the transcript is of small size, it contains a full representation of the sequences of the coding region of the mRNA[27].

Results similar to these have been obtained for sea urchin histone mRNAs[2,25] and neutral fixation of synonymous codon changes has been proposed to explain the interspecific divergence observed[25].

While the function of non-repeated DNA is still obscure, it is clear that the globin gene is conserved more stringently than is total non-repeated DNA. If this proves true for other proteins, it may be concluded that the selection pressure is less stringent on the bulk of the genome than on genes coding for polypeptides.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Heavy ION transfer reactions in nuclear astrophysical processes

AN appraisal of nuclear reactions in heavy ion systems which are known to be of importance in nuclear astrophysical processes indicated that reactions involving transfer of α particles could be of significant strength and hence should be included in reaction network studies which lead to the prediction of stellar abundances. The heavy ion systems of interest are $^{16}O + {}^{16}O$, $^{12}C + {}^{16}O$ and $^{12}C + {}^{12}C$. Preliminary results from a recent experiment (T. W. C., B. W. Hooton and G. Murray, to be published) have shown in the case of the $^{12}C + {}^{16}O$ system that, at energies of astrophysical importance, the channel leading to $^{20}Ne + 2\alpha$, which corresponds to the transfer on α particle, is of significant strength. This result influences predictions of the abundance of ^{20}Ne and that of elements depending on ^{20}Ne for their subsequent formation.

The purpose of this note is to identify for the heavy ion systems involved which reactions are likely to have a non-negligible rate. The astrophysical consequences of reaction channels formerly considered to be of negligible strength can only fully be appreciated when they are included in reaction networks which generate 'theoretical' abundances.

TABLE 1 Characteristics of α-transfer reactions in nuclear astrophysical processes

Thermonuclear epoch	E_0(MeV)	Region of interest			Energy (MeV)	Alpha transfer		Q optimum (MeV)
		Δ (MeV)	E_{min}	E_{max}	Coulomb barrier	Reaction	Ground state Q value (Mev)	corresponding to (E_{min} ro E_{max})
Carbon burning $^{12}C + {}^{12}C$	$2.42T_9^{2/3}$	$1.05T_9^{5/6}$	1.3	8.8	≈ 6	$^{16}O + {}^{8}Be$	-0.208	-0.14 to -0.98
Carbon-oxygen reactions $^{12}C + {}^{16}O$	$3.06T_9^{2/3}$	$1.19T_9^{5/6}$	1.9	10.7	≈ 8	$^{20}Ne + {}^{8}Be$	-2.639	-0.31 to -1.80
Oxygen burning $^{16}O + {}^{16}O$	$3.90T_9^{2/3}$	$1.34T_9^{5/6}$	3.8	13.1	≈ 10.6	$^{20}Ne + {}^{12}C$	-2.433	-0.24 to -0.82

At various evolutionary stages of some stars major concentrations of ^{12}C, ^{12}C and ^{16}O, and ^{16}O are believed to occur[1] in regimes corresponding to temperatures $T_9 = 1$ to 2 (and possibly up to 3.6), 1 to 3.6 and 2 to 3.6 respectively (where T_9 is in units of 10^9 K). The lower and upper limits in each case correspond generally to temperatures associated with hydrostatic and explosive burning stages respectively. Table 1 shows the most effective range of energies E_{min} to E_{max} (calculated in terms of E_0 and Δ as defined in ref. 2) promoting nuclear reactions under these conditions.

In Table 2 the reaction channels measured for the $^{12}C + {}^{12}C$, $^{12}C + {}^{16}O$ and $^{16}O + {}^{16}O$ systems are listed. These correspond to reaction channels expected to be most important from statistical evaporation theory following the formation of a compound nucleus. The sum of the principal components of the cross section is later called σ_R. The α-transfer reaction and equivalent three body breakup process (discussed below) are also given in Table 2; these are later referred to as σ_t and have not in general been measured.

I consider here only the importance of α-transfer reactions to the exclusion of other transfer processes since the extra nuclear binding associated with such α-cluster nuclei as ^{12}C and ^{16}O ensures for this reaction the most favourable or least negative Q value of all reactions involving single or few nuclear transfer. Furthermore the nature of these nuclei ensures for the transfer a good overlap of the nuclear wavefunctions (This is particularly true for $^{12}C \rightarrow Be_{g.s} + \alpha$ where the overlap is known to be large[3]).

In order to form a compound nucleus each heavy ion has to penetrate the Coulomb barrier of the other. The kinetic energy available to the ions in the upper region of astrophysical interest is comparable to the barrier height (Table 1, column 3). Under these circumstances transfer of an α particle from the one to the other heavy ion—a process which does not necessarily involve the interpenetration of the ions—is expected to be quite favourable and to become even more so as the kinetic energy of the ions is reduced to the lower region of astrophysical interest. Kozlovsky[4] has independently considered this possibility for the $^{12}C + {}^{12}C$ system. His calculations result in quite a small cross section of about 1% of σ_R for the α transfer in this case. On the other hand he indicates a very strong dependence of the transfer cross section on the binding energy of the transferred particle in the final nucleus. In the $^{16}O + {}^{16}O$, $^{12}C + {}^{16}O$ systems the final nucleus is ^{20}Ne in which the value of the α-binding energy is 4.73 MeV; this is much smaller than the value of 7.16 MeV for ^{16}O, the final nucleus in the case of $^{12}C + {}^{12}C$. We thus expect significantly higher cross sections for transfer in the $^{16}O + {}^{16}O$ and $^{12}C + {}^{16}O$ systems. A recent calculation (P. J. A. Buttle, private communication) for the $^{12}C + {}^{16}O$ system showed that the cross section for the α transfer $^{12}C(^{16}O, {}^{20}Ne)^{8}Be$ to the ground state of ^{20}Ne alone could be as high as 12 mb at 10 MeV. This is about 2–3% of σ_R. Recent theoretical estimates thus indicate that this reaction channel is of some significance in astrophysical processes.

The crux of the matter lies in the degree to which the cross section decreases as the bombarding energy (or temperature) decreases. It is at least conceivable that the latter falls less rapidly than σ_R and that over the region of astrophysical interest it makes an even more significant contribution to the overall reaction rate than the value of 2 to 3% quoted above would suggest, and, if so, should be included in reaction network studies from which abundances are calculated.

Alpha transfer in the three systems considered is also of considerable interest from the point of view of the dynamics of heavy ion interactions. It has been predicted and demonstrated[5] that for heavy ion reactions those reaction channels in which the distance of closest approach in the ingoing and outgoing channels is similar are favoured. The effect of this is to define a region of reaction Q values (Q-value window) around a value Q_{optimum} given by

Table 2 Components of σ_R and σ_t

	Principal compound channels	α transfer channel and equivalent three-body process
$^{12}C + {}^{12}C$	$^{20}Ne + \alpha$	$^{16}O + {}^{8}Be$
	$^{23}Na + p$	$^{16}O + \alpha + \alpha$
$^{12}C + {}^{16}O$	$^{24}Mg + \alpha$	$^{20}Ne + {}^{8}Be$
	$^{27}Al + p$	$^{20}Ne + \alpha + \alpha$
$^{16}O + {}^{16}O$	$^{28}Si + \alpha$	$^{20}Ne + {}^{12}C$
	$^{31}P + p$	

$$Q_{\text{opt}} \approx ((z_f Z_f / z_i Z_i) - 1) E_{CM}$$

in which reactions are favoured. z_t, Z_t are the atomic numbers of the heavy ions in the outgoing channel and z_i, Z_i refer to the ingoing channel. The last column of Table 1 indicates that at the upper end of the energy range of astrophysical interest the α-transfer reactions to the ground states of the final nuclei in all three systems should be favoured.

Although the discussion above has emphasised the possible importance of α transfer in the heavy ion systems of astrophysical interest it should also be mentioned that, in two of the systems considered, three-body breakup, namely

$$(1) \qquad {}^{16}O + {}^{12}C \rightarrow {}^{20}Ne + \alpha + \alpha$$

$$(2) \qquad {}^{12}C + {}^{12}C \rightarrow {}^{16}O + \alpha + \alpha$$

leads to the same final nucleus as that involved in α transfer. No direct measurements of this contribution to the total cross section in the cases discussed seem to have been made although it is the sum of this process and α transfer which, for example, determines the amount of ^{20}Ne produced in $^{16}O + {}^{12}C$ interactions and which modifies the number of α particles available for further interactions.

Of the three systems considered: oxygen-burning at temperatures characteristic of explosive nucleosynthesis has been shown[6] to produce the observed abundances of the nuclei between ^{28}Si and ^{42}Ca and carbon-burning regulates the amount of ^{16}O and ^{20}Ne available for late evolutionary stages; the importance of $^{12}C + {}^{16}O$ interactions during explosive nucleosynthesis has recently been dealt with by Woosley, Arnett and Clayton[1]. A literature search revealed no direct measurement of α-transfer cross sections for any of the reactions detailed above in the energy regions on or below the Coulomb barrier although an estimate has been made at two energies close to the barrier in the $^{16}O + {}^{16}O$ case[7]. This lack of data reflects the difficulty of detecting and identifying heavy ion species in the energy range close to 0.5 MeV per nucleon with conventional solid state detector telescope systems. A good case exists for a systematic study of all three reactions and their energy dependence not only for its astrophysical importance but also to provide measurements to clarify aspects of the heavy ion interaction.

This suggests that the reaction channels leading to ^{20}Ne which were discussed above should be included in calculations of the stellar ^{20}Ne abundance and related elemental abundances.

It is planned to extend the measurements as a function of angle and energy to compare the total yields of ^{20}Ne and ^{24}Mg. The measured yield of ^{24}Mg will provide a useful check on other method of measuring what is the principal contribution to σ_R. The systems $^{16}O + {}^{16}O$ and $^{12}C + {}^{12}C$ are also being studied.

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Association between the mid-winter stratospheric circulation and the first observation in the following summer of noctilucent clouds

Noctilucent clouds (NLCs) are seen mainly in the summer months at high latitudes over both hemispheres. They appear in a thin layer at a height of about 82 km in the mesopause region where temperatures are the coldest in the Earth's atmosphere. A temperature of 150 K or less at the mesopause has been shown, by rocket soundings in the presence of NLCs, to be a necessary condition for the existence of these clouds[1]. Rocket sampling experiments and ground-based observations indicate that NLC particles probably consist of a volatile substance, believed to be ice, coated on volatile nuclei that may be hydrated ions[2,3].

Thus two important variables, which help to determine whether the formation of NLCs will occur, are the water vapour content and temperature. Both parameters depend on the mesospheric circulation, whose main features at a particular latitude vary seasonally. In consequence it has been suggested that observations of NLCs might be used as a guide to the mesospheric circulation. For example, the onset of NLCs in early summer might relate to the development of the summertime circulation pattern in the mesosphere.

The first observations of NLCs each year fall into three categories:

(a) Mid-winter, for example January 5, 1964 at Lerwick, Scotland[4]. There are few of these observations.

(b) Spring equinox. Occasional reports during March and April have been noted. However they are confined to data from North America and the USSR.

(c) Early summer. Western European data regularly start in late May or early June.

Observations in category (a) have been shown to be correlated with record temperature and wind observations in the lower stratosphere[5]. Although it is generally assumed that the wintertime mesopause is always much warmer than in summer, there have been observations by the grenade technique in mid-winter of mesopause temperatures as low as 148 K[6]. These were also associated with similar extreme disturbances in the stratosphere. On such occasions temperature changes of opposite sign appear to take place at adjacent levels. Thus the mesosphere may cool to below mean summertime temperatures while the stratosphere is undergoing a 'warming.' These anomalous mid-winter phenomena are temporary, lasting perhaps for a few days, before the circulation starts reverting to the normal wintertime pattern.

Category (b) observations are difficult to explain and are not discussed in this paper. It is hard to understand why there should be no equinoctial observations for Western Europe if those from the USSR and North America are valid.

The observations in category (c) are of particular interest. They are from a series of papers published in the Meteorological Magazine by Mr J. Paton of the University of Edinburgh[7]. The data have been subject to strict criteria before acceptance and have been recorded by an experienced group of observers. As the observations are limited to Western Europe between latitudes 50° and 60° N, any variations due to latitudinal or longitudinal differences are greatly diminished. These data demonstrate a surprising feature which is shown in Fig. 1. This is the tendency for the first observation of NLCs to be earlier in those summers following a major stratospheric warming (MSW) in the previous winter. The disturbance to the mid-winter stratospheric circulation is represented in the figure by the maximum altitude (in km) reached at the North Pole by the 10 mbar surface during the months of December and January. These data are taken from the reports published by the Staff of the Stratospheric Research Group at the Free University of Berlin. The 10 mbar surface rose to heights ≥30.80 km only for the seven years when an MSW occurred in mid-winter (1960, 1963, 1966, 1968, 1970, 1971, 1973). In each case a reversal of the latitudinal temperature gradient at 10 mbar took place (warm air replaced cold air over the pole) and the westerly circulation collapsed to be replaced, for at least a few days, by a weak easterly flow.

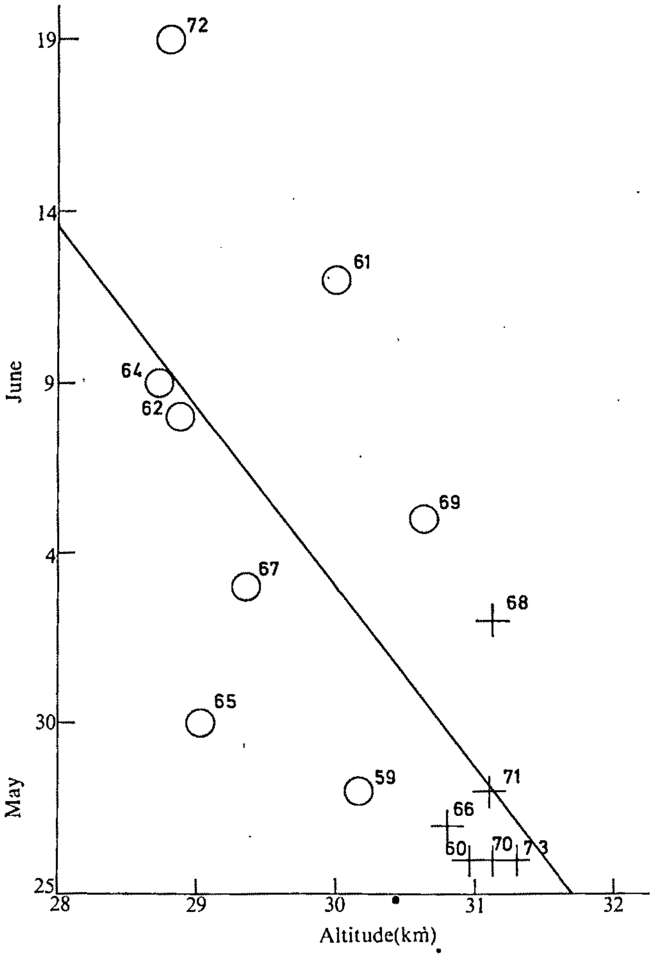

FIG. 1 Comparison of the date of the first NLC observation with the maximum height (km) reached at the North Pole by the 10 mbar surface during the months of December and January the preceding winter. ○, Year without MSW; +, year with MSW.

In six out of these seven years, the first NLC observation was never later than June 2. The exception is 1963 when the first observation was on June 28. This datum point is excluded from Fig. 1 on the following grounds:

(a) The observers in 1963 were not as numerous or as familiar with the clouds as today—regular observations to see whether NLCs were present did not start until the IQSY in 1964.

(b) A big problem in late May and June 1963 was the poor visibility. Much cloud and rain were reported by the relevant meteorological stations at that time. Leuchars, one of the most likely NLC reporting stations, recorded twenty-six cloudy nights in June. A doubtful observation of NLCs mixed with cirrus and patchy hill fog was made on May 29—this was rejected for publication.

For the six years of data for which an MSW occurred during the preceding winter, the mean date of the first NLC observation was May 28 with a standard deviation of only 2.7 d. For illustration, the least-squares technique was used to fit to the data a straight line

$$y = (19.6 \pm 3.7) - (5.3 \pm 1.6)x$$

where y is the date of the first NLC observation in days after May 25 and x is the maximum height of the 10 mbar surface in km above 28 km.

These results are surprising. It was not expected that such a clear association would be apparent between events occurring four to six months apart and at different levels of the upper atmosphere. Following on from these results it was thought that there might be an association between the stratospheric circulation at the final spring warming when the changeover from winter to summer conditions occurs and the first reports of NLCs. Until 1964 it was possible to classify these final warmings at 10 mbar into early or late types; however, no early final warmings occurred after 1964 until 1972 (ref. 9). Further, the mean monthly 10 mbar heights at the pole for the months of May were found to show no correlation with the presence the previous winter of an MSW or the first reports of NLCs.

Thus in the lower stratosphere the effects of an MSW seem to be smoothed out by the end of March whereas at the mesopause, effects are still visible at the end of May. These results emphasise the importance of studying the dynamic interactions between the stratosphere and mesosphere and of considering the phenomenon of a stratospheric warming as affecting the mesosphere as well as the stratosphere.

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Electric cloud and weather modification with intense relativistic electron beams

THE problems confronting weather modification by seeding methods are well known and centre on the dispersion of the seeding agent over a large air volume. In a different method for artificial cloud modification proposed by Vonnegut and Moore[1] electric charges are released by corona discharges into updraughts from the ground by a system of wires at a high electric potential. In this method the electric charges are much more rapidly dispersed, due to a rather large ion mobility in a thundercloud electric field, than is otherwise possible for seeding agents dispersed by turbulent convection. But the system of ground wires seems to be impractical for a weather modification technique with the demand for high mobility.

I therefore propose here an alternative method of electric cloud and weather modification based on the projection of a rapid sequence of intense relativistic electron beams into the atmosphere. The maximum range in the atmosphere of relativistic electrons is given by the radiation length $l_0 \sim$ 280 m and which is rapidly approached for electron energies in excess of 50 MeV. A beam of electrons would normally disperse rapidly in the radial direction due to multiple scattering. This however, is not the case for an intense beam of relativistic electrons which is confined by its own magnetic field. Beams up to 10^6 A and with electron energies of many million electron volts lasting up to 100 ns have been already produced by the combination of a Marx surge generator with a Blumlein transmission line, connected to a field emission diode. The total beam energies so far achieved reach the value of megajoules. If such a beam is projected into the atmosphere it will not disperse radially but rather will produce a channel of hot air, with a maximum channel length equal to the radiation length in air. If now a second beam is projected into this channel after a time lapse which is equal to the time for the air to expand in the channel heated by the first beam and which is given by $\tau_{\mathrm{exp}} \sim r/a$, where r is the beam radius and a the velocity of sound in the heated air, then the second beam will in part propagate through air of reduced density due to the thermal expansion caused by the first beam. If for example, the first beam heats the channel up to $\sim 10^4$ K the velocity of sound would be $\sim 10^5$ cm s^{-1}, and if the original beam radius is $r \sim 0.1$ cm the time for expansion would be $\tau_{\mathrm{exp}} \sim 10^{-6}$ s. Because of the reduced attenuation the second beam will propagate further than the first beam. A third beam will propagate even farther, and so on. A sequence of beams rapidly projected along the same path will therefore tunnel into the atmosphere much deeper than otherwise possible with only one beam. Such a rapid sequence of beams can be obtained by a parallel arrangement of Marx generators to be discharged in a programmed sequence by triggered spark gap switches over the same Blumlein line and discharge diode.

I define the ratio $T_0/T = \kappa$ where T_0 is the air temperature under normal conditions and T the temperature in the channel heated by the beam. For $T \sim 10^4$ K, $\kappa \sim 3 \times 10^{-2}$. With a beam channel volume of $\pi r^2 l_0 \sim 10^3$ cm^3 a beam energy $\epsilon \sim 10^4$ J would raise the temperature to $T \sim 10^4$ K. The first beam pulse will penetrate the distance $\delta_1 = l_0$. The second beam will add to this the penetration length $\delta_2 = (1-\kappa)l_0$, the third will add $\delta_3 = (1-\kappa)\,\delta_2 = (1-\kappa)^2\, l_0$, and the nth beam will add $\delta_n = (1-\kappa)\,\delta_{n-1} = (1-\kappa)^{n-1}\, l_0$. The total penetration length is therefore given by

$$l_n = \sum_{n=1}^{n} \delta_n = l_0 \sum_{n=1}^{n} (1-\kappa)^{n-1} = [1-(1-\kappa)^n]\,l_0/\kappa.$$

For $n \rightarrow \infty$, $l_\infty = l_0/\kappa$. For $l_0 = 2.8 \times 10^4$ cm and $\kappa = 3 \times 10^{-2}$, $l_\infty \sim 10$ km. For $n = 10$ pulses the length would be $l_{10} = 2.4$ km. In this case each beam pulse delivers $\sim 10^4$ J such that the total energy delivered in ten subsequent pulses, with a time lapse of $\sim 10^{-6}$ s between the pulses, is $\sim 10^5$ J. The total time to discharge ten subsequent pulses is $\sim 10 \times 10^{-6}$ s $= 10^{-5}$ s and is short enough to prevent the beam channel from being destroyed by either heat conduction or wind motion. A capacitor bank of 10^5 J has an approximate weight of several tons. The capacitor bank could be thus mounted on a heavy truck or airplane and charged up by a combustion engine, making the electron beam machine mobile, a desirable requirement if the beam is to be employed for cloud or weather modification.

This method promises the following: (1) It permits the disposal of a large negative electric charge into a chosen volume of the atmosphere or of a cloud. (2) The intense relativistic electrons release within the atmosphere a large X-ray flash producing many pairs of positive and negative ions. (3) The beams create within the atmosphere channels of high electrical conductivity.

The implications of these effects to potential cloud and weather modification are manifold. The most important ones seem to be: (1) The local release of electric charges can result in greatly increased electric fields within a certain cloud region leading to enhanced cloud droplet coalescence resulting in increased precipitation. (2) The release of electric charges in an updraught may accelerate the thundercloud electrification by the Vonnegut-feedback mechanism[2] and conceivably in this way also increase precipitation. (3) The creation of a large number of ion pairs will increase the electric conductivity of the air which could be used for lightning supression. (4) The creation of long channels of high electrical conductivity could be used to trigger artificially cloud-cloud or cloud-ground discharges.

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Astrophysical fuel-coolant interactions

If a hot liquid comes into contact with a cold vaporisable liquid a violent explosion may occur[1-5]; such interactions are often called fuel-coolant interactions (FCIs). In this letter we apply FCI theory to the problem of matter and antimatter interacting on an astronomical scale.

Buchanan and Dullforce[6] explain FCIs as follows: (1) an initial perturbation causes a small vapour bubble to form at the fluid interface; (2) as the vapour condenses the bubble collapses and, because of local asymmetry, a jet of cold liquid forms; (3) the jet penetrates the hot fluid and disintegrates; and simultaneously (4) the jet material is heated; (5) as the jet is vaporised a new bubble forms and expands. This process continues cyclically through stages (2)–(5). D. J. Buchanan (to be published) has shown that the initial perturbation is damped out if the external pressure is greater than some threshold value, and recently we[7] have used this fact to suggest that volcanic hydroexplosions[8] in the form of FCIs[9] do not occur at sea depths greater than some critical value.

Similar arguments can be applied to the problem of interacting clouds of matter and antimatter by noting that the FCI model can be generalised to describe a fluid interface instability of wider application. Conditions necessary for this instability are an energy releasing reaction (for example through the contact of hot and cold liquids), the production of an intermediate phase which is then condensed (for example the vapour bubble), and an asymmetry which causes intermixing by jet penetration and thus further energy releasing reactions. The energy releasing reaction need not be thermal and the intermediate phase need not be a vapour; for example, the energy releasing mechanism might be chemical.

Alfvén[10] has suggested that regions of matter and antimatter could be separated by a thin Leidenfrost layer of radiation sustained[11,12] by gradual annihilation. The following instability can be postulated. A perturbation locally decreases the thickness of the Leidenfrost layer, thus the annihilation rate increases and a radiation bubble forms. Initially the bubble expands and, provided the radiation escapes, it then collapses. Departure from mirror symmetry can result in the formation of a jet of matter, say, if the bubble is in the matter region; if this jet penetrates the antimatter region further annihilation will occur and another bubble will form. Significant differences in, for example, density, viscosity or magnetic field strength constitute departures from symmetry. Thus the possibility exists of explosive mixing of matter with antimatter analogous to FCIs.

If the ambient pressure at the interface is greater than some threshold value, P_{th}, then the initial perturbation is damped out. According to Buchanan (to be published)

$$P_{\text{th}} = (3\alpha^3)/1 + \alpha + \alpha^2 P_i \qquad (1)$$

where P_i is the pressure in a bubble at its formation in any cycle and α is given by

$$\alpha^3 = (3/16)\beta d_c{}^2 L_c. \qquad (2)$$

Here β is the fraction of the jet that is annihilated and d_c and L_c are dimensionless non-negative numbers less than unity which characterise the jet[6,13] and are determined by the degree of local asymmetry. For perfect symmetry d_c and L_c are both zero.

If ρ is the density of a jet of volume V then the energy released by annihilation is $2\rho c^2 V$ giving an initial pressure of $(4/3)\rho c^2$ and so for $\beta = 1$

$$P_{\text{th}} \simeq \tfrac{3}{4} d_c{}^2 L_c \rho c^2 \qquad (3)$$

If the ambient pressure is greater than P_{th} the instability is suppressed.

As an illustration of the consequences of equation (3) consider a self-gravitating sphere of mass M and radius R with an antimatter core (or *vice versa*). The central pressure pressure P_c and the mass are given by[14]

$$P_c = W_n M^2 G/R^4 \qquad (4)$$

and

$$M = C_n \tfrac{4}{3}\pi R^3 \rho \qquad (5)$$

where ρ is the central density, G is the gravitational constant, and W_n and C_n are dimensionless numbers depending on the polytropic constant. From equations (3), (4) and (5) we deduce that the matter/antimatter interface is stable to an 'intermediate phase instability' if the Schwarzschild number $S(= 2GM/Rc^2)$ satisfies

$$S > (9/8)(d_c{}^2 L_c/\pi W_n C_n). \qquad (6)$$

If the adiabatic constant $\gamma = 5/3$ then $n = 3/2$ and $W_{3/2} C_{3/2} = 0.129$ and for $\gamma = 4/3$, $n = 3$ and $W_3 C_3 = 0.213$. Hence $W_n C_n$ is of order 10^{-1} for likely values of γ. The largest likely values of d_c and L_c are those appropriate to bubble collapse adjacent to a solid wall,[13] namely about ¼ and ½ respectively. Thus the inequality (6) becomes

$$S \gtrsim 5 \times 10^{-2} \qquad (7)$$

This value of the Schwarzschild number is large compared with those normally encountered in astrophysics; for example, both the Sun and the galaxy have $S \simeq 10^{-6}$. If the radius of the Sun were 70 km, like that of a neutron star, then condition (7) would just about be satisfied. When condition (7) is satisfied we have the interesting possibility of a region of antimatter existing at the centre of a large region of matter, the whole system being stable against dynamic mixing arising from this type of instability.

Other examples, such as the collision of two bodies under gravitational attraction or through their random velocities, result in a similar condition involving the Schwarzschild number, but in these cases the high pressure generated on impact is only a transient feature, and cannot permanently inhibit the instability.

The immediate implication of this admittedly crude model is that the stability of the Leidenfrost layer postulated by Alfvén depends critically on the Schwarzschild number and the degree of symmetry at the matter/antimatter interface.

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Atmospheric carbon dioxide levels as indicated by the stable isotope record in wood

THE concentration of CO_2 in the atmosphere has increased from about 290 parts per million (p.p.m.) by volume in 1900 (ref. 1) to 323 p.p.m. in 1972 (ref. 2) and this increase is generally held to be due chiefly to combustion of fossil fuels. Indeed by 1970 this artificial mechanism had introduced to the atmosphere an amount of CO_2 equivalent to 22% of the natural or pre-industrial atmospheric inventory[3]. About half of the added CO_2 seems to have been transferred to the other major reservoirs of the dynamic carbon cycle, the oceans and biosphere. Before examining the threat to global climate and marine life[4], future CO_2 levels from this source must be predicted. Before such extrapolations can be confidently applied, however, we must fully understand the past response of the atmosphere to fossil CO_2 emission. Since direct measurements of atmospheric CO_2 are either not available or considered unreliable indirect methods of assessing the CO_2 buildup must be invoked. Here a novel and potentially valuable approach is presented.

The carbon isotopic composition of fossil CO_2 can be distinguished from that of natural atmospheric CO_2 in two significant ways; (1) the radioactive isotope of carbon, ^{14}C, present as one part per 10^{12} in atmospheric carbon, is totally absent in fossil carbon because of the rapid radioactive decay of ^{14}C (half-life 5,730 yr) relative to the typical age of fuel deposits; (2) the secondary stable isotope of carbon, ^{13}C, on average about 1.1% of nature's total carbon, is 2% less abundant in terrestrial plants and their fossil remains (coal, petroleum, natural gas) than in atmospheric CO_2 (ref. 5; Fig. 1). The burning of fossil fuels, therefore, has released to the atmosphere increasing amounts of both ^{14}C and ^{13}C-depleted CO_2. It seems reasonable to suppose that both the $^{14}C/^{12}C$ and $^{13}C/^{12}C$ ratios of atmospheric CO_2 will have decreased in sympathy with the addition of lighter CO_2. Furthermore, such trends should be permanently recorded in the annual tree rings of wood which, in effect, derive their carbon directly from the ambient atmosphere. Indeed the decline of the $^{14}C/^{12}C$ ratio is well documented. Extrapolation from ^{14}C data to levels of fossil CO_2 is, however, of limited value since there is evidence that natural variations in $^{14}C/^{12}C$ have accompanied the artificial dilution effect[6]. Until now there has been no attempt to evaluate the stable isotope record. If isotopic fractionation during photosynthetic assimilation of atmospheric CO_2 by growing wood has remained constant, $^{13}C/^{12}C$ assay of tree rings of known age can in theory yield additional information on the past levels and isotopic composition of atmospheric CO_2.

We have measured the $^{13}C/^{12}C$ ratios of individual rings from two trees: (1) an oak (*Quercus robur*) planted 1848, felled 1968, from the Forest of Dean (51°48′N, 2°37′W), England and (2) a European larch (*Larix decidua*) planted 1892, felled 1972, from Glengarry Forest (57°01′N, 4°51′W), Scotland. To minimise isotopic fractionation effects during experimentation, the wood samples were not subjected to any chemical treatment prior to conversion to CO_2 for isotope ratio measurement. The $^{13}C/^{12}C$ ratios were determined using a double collecting, 90° deflection, 6 cm radius Micromass 602B mass spectrometer and the results expressed to a precision of $\pm 0.1‰$ (2σ) relative to the primary standard, PDB belemnite limestone. On this scale the isotope data are quoted as $\delta^{13}C$, the per mille deviation of sample $^{13}C/^{12}C$ from that of the standard, by the expression[7]

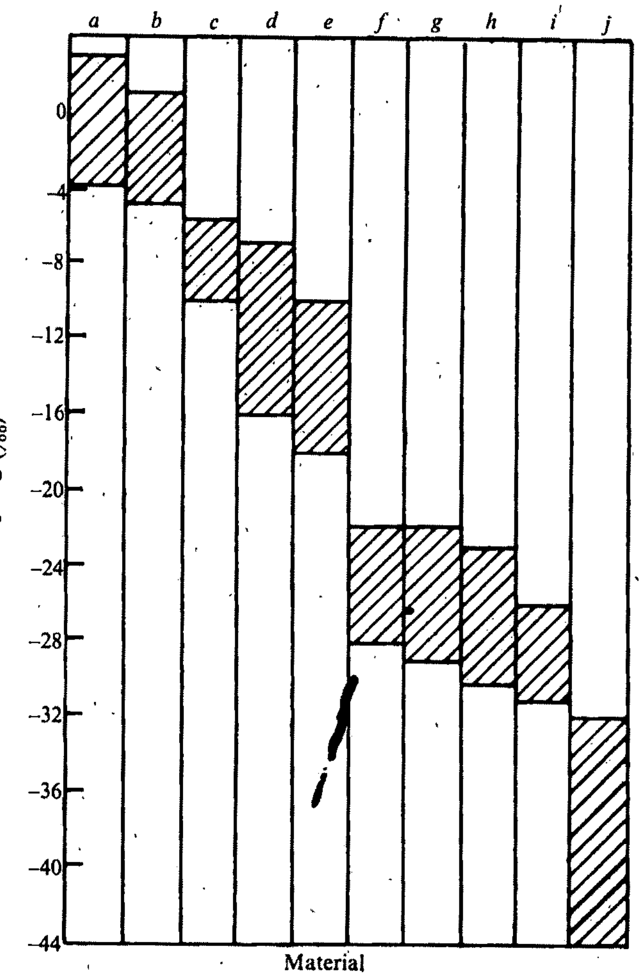

FIG. 1 Carbon isotope ratios of various carbonaceous materials. *a*, Carbonates; *b*, volcanic CO_2; *c*, atmospheric CO_2; *d*, marine plants; *e*, marine organisms; *f*, coal; *g*, land plants; *h*, liquid hydrocarbons; *i*, organic sediments; *j*, natural gas.

$$\delta^{13}C_{(\text{sample})} = \frac{{}^{13}C/{}^{12}C_{(\text{sample})} - {}^{13}C/{}^{12}C_{(\text{standard})}}{{}^{13}C/{}^{12}C_{(\text{standard})}} \times 1{,}000‰.$$

To smooth the distribution of results a 10-yr running mean was calculated for each series and the two curves plotted as shown in Fig. 2. Before detailed discussion of these results it should be noted that the systematic difference (~1.1‰) between the early sections of both curves can reasonably be attributed to a smaller fractionation effect in larch wood synthesis.

Both trees show a rapid decline in $\delta^{13}C$ during the early years of this century amounting to 1.5‰ from 1900–20 for the oak and 1.1‰ from 1905–21 for the larch (1.2‰ for the oak). The two curves also exhibit similar tends after 1944, rising to a more positive $\delta^{13}C$ by 1958 and then declining rapidly to 1964. Between 1922 and 1944 the curves are dissimilar.

Assuming constant CO_2 fractionation factors of (1) −19.9‰ and (2) −21.0‰ between the atmosphere and (1) larch and (2) oak respectively[8] there has been an average decline in atmospheric $\delta^{13}C$ of 1.7‰ from −4.9‰ in 1900 to −6.6‰ in 1964. If we consider the 1964 CO_2 concentration of 316 p.p.m.[9] ($\delta^{13}C_{1964} = -6.6‰$) as the result of a combination of the 1900 CO_2 concentration ($\delta^{13}C_{1900} =$

$-4.9‰$) and additional fossil CO_2 ($\delta^{13}C_f = -26.0‰$) then the 1900 CO_2 concentration can be calculated from

$$[CO_2]_{1900} = \frac{[CO_2]_{1964}\,(\delta^{13}C_{1964} - \delta^{13}C_f)}{(\delta^{13}C_{1900} - \delta^{13}C_f)}$$
$$= 290\cdot5 \text{ p.p.m.}.$$

This figure is in excellent agreement with the generally accepted estimate of 290 p.p.m.[1] Although this ideal consistency of the model may be coincidental, similar calculations are especially instructive for the earlier years of this century.

Both trees show a decline in $\delta^{13}C$ of 1.5‰ between 1900 and 1920. Such a decrease could not have been caused even by complete retention of all the fossil CO_2 introduced to the atmosphere from 1900–20 (ref. 10) (a CO_2 input equivalent to 7.9 p.p.m.); that is

$$\delta^{13}C_{1920} = \frac{\delta^{13}C_{1900}[CO_2]_{1900} + \delta^{13}C_f([CO_2]_{1920} - [CO_2]_{1900})}{[CO_2]_{1920}}$$
$$= -5\cdot5‰ \text{ (a decrease of only } 0\cdot6‰)$$

A 1.5‰ decline due to an addition of CO_2 of $\delta^{13}C = -26.0‰$ corresponds to a calculated $[CO_2]_{1920}$ concentration of 312.7 p.p.m., an increase of 22.2 p.p.m. over the 1900 figure of 290.5 p.p.m. as compared to a total fossil CO_2 addition of 7.9 p.p.m. Intriguingly, this figure of 312.7 p.p.m. is fair agreement with some early and hitherto disputed direct measurements of atmospheric CO_2 concentrations which nevertheless were considered sufficiently reliable to be included by Callendar[1] in his summary of twentieth century levels. The implication therefore is that, during the early decades of this century, there was an appreciable additional source of CO_2 of $\delta^{13}C$ similar to that of land plants (-25 to $-26‰$). The possibility of a CO_2 contribution from volcanic emission or from warming of the oceans ($0 \leq \delta^{13}C \leq -5‰$) is apparently excluded. A likely source of this additional CO_2 could have been the oxidation of organic litter of terrestrial land plant composition accompanying enlargement of the world's cultivated farmland at the expense of forests and grassland. In support of this premise is the estimate by Revelle[11] that the observed increase in arable land of $\sim 1.6 \times 10^6$ million square miles corresponds to a humic CO_2 input of $\sim 10^{17}$g CO_2, a figure of exactly the required order of magnitude.

It is evident that the rapid increase in CO_2 inferred above did not continue until the present. During the period 1944 to 1958 in particular both trees record trends to more positive $\delta^{13}C$. It is likely then that during recent decades an additional influence has become dominant and that, through partial compensation of the fossil CO_2 flux and resultant $\delta^{13}C$ levels, it has stabilised the atmospheric CO_2 burden. The most probable causes of this latter effect are enhanced CO_2 exchange with and uptake by the oceans, perhaps in sympathy with the observed 0.1–0.2° C drop in mean global temperatures since 1940 (ref. 12), or alternatively an increase in the size of the terrestrial biomass in response to the rise in atmospheric CO_2 levels. Whatever the responsible mechanism it is apparent that an important stabilising influence has been exerted. The discrepancies between the curves for the 1922–44 interval may serve to illustrate the dangers of extrapolation from results which could be subject to local effects. It is nevertheless possible that the data from this period do indeed reflect local differences in response to the transition between two competing mechanisms. During the past 15 yr the decline in $\delta^{13}C$ is in line with the reported direct measurements of the increase in atmospheric CO_2.

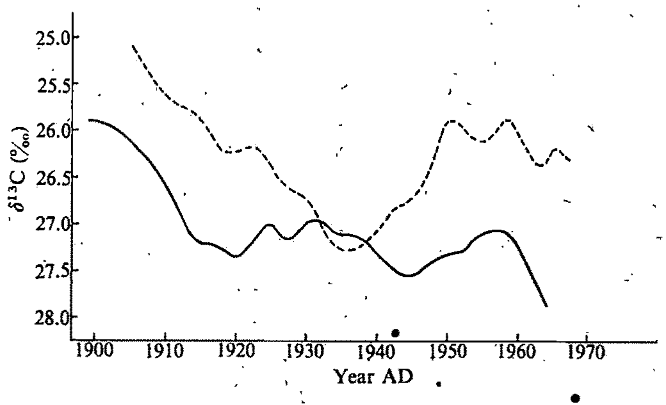

FIG. 2 Temporal variations of carbon isotope ratio observed in twentieth century larch (-----) and oak (———) wood.

The assumption of constancy of fractionation between atmospheric CO_2 and growing wood during the past 70 yr may perhaps be open to doubt. Craig[13], however, has reported that the long-term trend of wood $\delta^{13}C$ from 900 BC to 1600 AD was constant to within 1‰. Although minor variations could be induced by the effects of varying external conditions on the assimilation and respiratory process of a tree, it seems most improbable that a 2‰ decrease in wood $\delta^{13}C$ could be attributed to such factors particularly in view of the considerable magnitude of the fossil CO_2 injection considered here. Furthermore, it is of interest that Dequasie and Grey[8] found a similar rapid 1.3‰ decrease in United States poplar wood $\delta^{13}C$ from 1912–27 and an overall decrease of 2.3‰ by 1966.

While more data are required for a precise assessment, the results of this preliminary study imply (1) an additional source of CO_2 of $\delta^{13}C \sim -26‰$, in conjunction with fossil fuel combustion, contributed to rapidly increasing atmospheric CO_2 concentrations during the first 20–30 yr of this century and (2) variations in exchange and removal rates of CO_2 across the respective atmosphere/biosphere/ocean interfaces have occurred in view of the irregular and somewhat unexpected trends of $\delta^{13}C$ during the past 40 yr. Therefore, in view of the apparent complexity of the interactions and responses involved, prediction of future CO_2 levels probably cannot be performed with confidence. But continued research along these lines should assist towards the evaluation of the controlling geophysical and geochemical parameters within the carbon cycle and eventually towards a more definite assessment of the likely fate of fossil CO_2.

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BIOLOGICAL SCIENCES

Recovery of Yeast from Transient Inhibition of DNA Synthesis

IN the simple eukaryotic cell *Saccharomyces cerevisiae* nuclear division, cell division and the start of new budding cycles depend on DNA synthesis[1]. Protein synthesis is required for the recovery of new budding and cell division after transient exposure to hydroxyurea, a specific reversible inhibitor of DNA synthesis[2]. In this report I have attempted to answer two questions. (1) Does recovery of nuclear division require protein synthesis? (2) Does recovery of DNA synthesis itself require protein synthesis?

To answer the first question a log phase culture was exposed to hydroxyurea to arrest cells with undivided nuclei in the necks between mother cells and buds. In a portion of the culture from which hydroxyurea was removed and replaced by an inhibitor of protein synthesis, anisomycin, the nuclei did not divide (Fig. 1). Therefore, protein synthesis is needed for the recovery of nuclear division. In conjunction with the previous findings[2] this indicates that during exposure to hydroxyurea some of the proteins needed for the execution of all events dependent on DNA synthesis are not made (or do not remain stable).

When protein synthesis is blocked after exposure to hydroxyurea nuclear division is prevented, but the complete round of DNA synthesis required for nuclear division might not be prevented. To test this possibility a log phase culture was exposed to hydroxyurea to yield cells with nuclei in the neck. Protein synthesis was blocked with anisomycin, and hydroxyurea was removed. After 1.5 h, hydroxyurea was reintroduced to part of the culture (to prevent further DNA synthesis), anisomycin was removed from the whole culture and nuclear division was monitored (Fig. 2). If the DNA synthesis required for nuclear division occurred in the presence of anisomycin, then reintroduction of hydroxyurea when the antibiotic was removed would not prevent subsequent nuclear division. I found that when the anisomycin was removed, the nuclei divided with the same kinetics whether or not hydroxyurea was present. The result was the same with initial exposures of 2.5 and 3.5 h to hydroxyurea and when the concentration of anisomycin was 10^{-4} M (three times the amount completely inhibiting protein synthesis more than 90% within 5 min) or 3×10^{-4} M. I therefore conclude that blocking protein synthesis when hydroxyurea is removed does not prevent a full round of DNA synthesis.

This conclusion was confirmed by experiments in which DNA synthesis was followed by measuring the incorporation of radioactive precursors. On six occasions portions of a steady state labelled log phase culture were exposed to anisomycin or first transiently exposed to hydroxyurea and then exposed to anisomycin. Exposure times to hydroxyurea varied from 2 to 3.5 h and the concentration of anisomycin varied from 10^{-4}M to 3×10^{-4} M. The residual increase of DNA in the presence of anisomycin was measured as described in the legend of Fig. 3 (my unpublished results). For cultures pretreated with hydroxyurea the average increase was 85%: this was the value predicted, since 33% of the cells were engaged in DNA synthesis when hydroxyurea was added[2]. On average, the genomes of these cells were half replicated, which accounts for about 17% of the DNA in the culture. Since this DNA did not replicate again the expected value was 100%–17% or 83%. By the same reasoning a 17% increase was expected for cells not previously exposed to hydroxyurea. The value found was 24%, which was high. This may be due to continued mitochondrial DNA synthesis and the experimental error inherent in measuring DNA in yeast.

Special proteins must be made for the initiation of DNA synthesis. They are made and remain functional during exposure to hydroxyurea, as demonstrated by the following observation. When protein synthesis was inhibited with anisomycin before the start of DNA synthesis in synchronised cells, initiation of DNA synthesis was prevented. If, however, DNA synthesis was first prevented by hydroxyurea, and anisomycin was added when hydroxyurea was removed, DNA was synthesised in the absence of protein synthesis (Fig. 3).

In the normal yeast cell cycle protein synthesis is required for initiation but not continuation of DNA synthesis[3,4]. My results show that during a 3.5 h exposure to hydroxyurea,

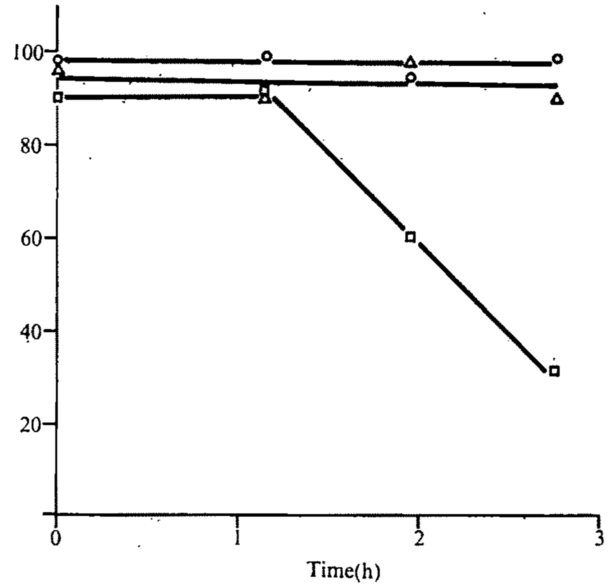

Fig. 1 Effect on nuclear division of adding anisomycin after reversible inhibition with hydroxyurea. For all experiments the yeast strain and growth medium were as described previously[2]. Unless otherwise stated, the concentrations of hydroxyurea and anisomycin were 0.075 M and 3×10^{-4} M, respectively. For all medium changes, cells were collected on filters[2], washed with 3 volumes of prewarmed medium and resuspended in prewarmed medium. The same medium was used for washing and resuspension. Hydroxyurea was added to a log phase culture. After a 3.5 h incubation (time 0 on the graph) cells were transferred to fresh growth medium (□) or to medium containing anisomysin (△), while incubation in the presence of hydroxyurea was continued for a third sample (○). Duplicate 5 ml portions were removed for nuclear staining[15].

the proteins for DNA synthesis are made, remain functional, and can be expressed in a full round of DNA synthesis in the absence of concomitant protein synthesis. This is consistent with and confirms the earlier results[3,4]. Functional tests (mitosis or division) have detected failure to replicate the last 3% and 0.5% of the HeLa cell[5] and *E. coli* genomes[6], respectively. The nuclear division assay used here is analogous and demonstrates completion of DNA synthesis independently of the direct measures of DNA used by Williamson[3].

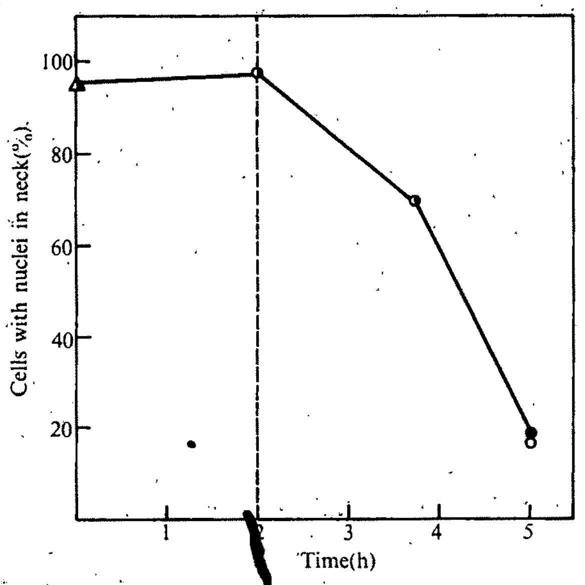

Fig. 2 Effect on nuclear division of successive exposures to hydroxyurea, anisomycin and hydroxyurea. Hydroxyurea was added to a log phase culture. After a 3.5 h incubation (time 0 on the graph) a sample was removed for nuclear staining (△) and the remaining cells were transferred to medium containing anisomycin. At the time indicated by the line a sample of the culture was transferred to medium containing only hydroxyurea (●) and another was transferred to fresh growth medium lacking inhibitors (○). Samples were removed for nuclear staining[5].

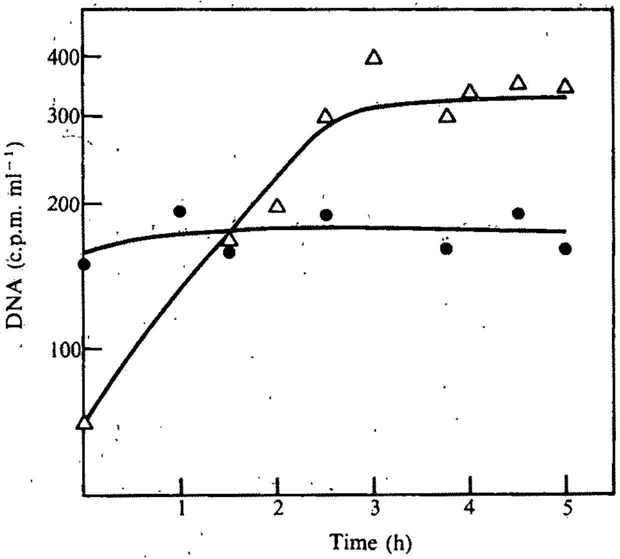

Fig. 3 Effect of anisomycin on the initiation of DNA synthesis in synchronized cells pretreated with hydroxyurea. A culture was synchronised by the starvation method of Williamson[16] and inoculated at 5×10^5 cells ml^{-1} into growth medium containing ^{14}C-uracil (0.3 μCi ml^{-1}, specific 10 mCi mmol^{-1}). At 30 min before the second cycle of DNA synthesis (and budding) anisomycin was added to one portion of the culture and hydroxyurea to the other. After a 2 h incubation (time 0 on the graph) the cells incubated in the presence of hydroxyurea were transferred to medium containing anisomycin (△) while the other portion was incubated further with anisomycin (●). Duplicate samples of 2 ml were analysed for radioactivity in DNA as previously described[2].

The initiator proteins, presumably inactivated in the conditional mutant used by Hereford and Hartwell[4], are shown here to be stable for 3.5 h in wild type cells. This is longer than the 3 h half life estimated for Chinese hamster cell initiator(s)[7].

The ability to execute a complete round of DNA synthesis in the absence of concomitant protein synthesis is unusual for a eukaryotic cell[8–10]. Studies with the slime mould *Physarum polycephalum* indicate that the replication of 'early replicating DNA' is required for the synthesis of proteins which start the replication of other DNA[10]. Thus there is a causally connected ordered sequence of genome replication. This is obviously not true for DNA synthesis in yeast. A similar type of sequence may, however, exist for events in the 'DNA-division' cycle of yeast. The programmes leading to events which require DNA synthesis begin early in the cell cycle[11,12]. The work with hydroxyurea shows that in its presence all the proteins needed for a full round of DNA synthesis are made. Some proteins, however, are not made for the events which depend on DNA synthesis (that is, cell division, new budding[2] and nuclear division). When hydroxyurea is removed, DNA is replicated and the proteins for the events which require DNA synthesis are made. This suggests, but does not prove, that the synthesis of proteins in the programme leading to DNA-dependent events are triggered by DNA synthesis which is triggered by the proteins made in the presence of hydroxyurea.

The unusual properties of DNA synthesis and nuclear composition (lack of lysine-rich histone from the nucleochromatin network[13,14] and loose DNA packaging) might be clarified by studying the effects of inhibiting DNA synthesis on yeast 'histone' synthesis.

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Do D-Glucosamine and D-Galactosamine Form Part of Specific Receptor Sites for Competence Substance on Cell Wall of *Pneumococcus*?

D-GLUCOSAMINE and D-galactosamine probably form a part of the specific receptor sites for the competence substance on the cell wall of *Pneumococcus*, since these amino sugars completely inhibit the binding of the competence substance to the cell surface receptors, and, thus, the induction of competence in a genetically transformable pneumococcal strain.

The importance of the architecture of cell surfaces in determining biophysical interactions with other cells, with viruses, and with naked DNA molecules which may lead to gene flow from a donor to a recipient cell, has long been known, but information about the molecular mechanism of such interactions is still rather scarce. A specific surface structure (a different antigen) in each case differentiates male from female bacteria, cells which adsorb phage from those which do not, competent from non-competent populations of transformable bacteria, and malignant from normal cells. In all these cases the introduction of a new component seems to alter the surface charge of the cell bearing it, thus potentiating the various macromolecular interactions.

The significance of glycosyl residues seems to be that they confer a specific recognitional role on biopolymers. Antibody proteins of higher animals interact specifically with polysaccharides. A similar type of interaction has now been found with phytohaemagglutinins (PHA). PHA binds to carbohydrate structures on the cell surfaces and agglutinates erythrocytes and other types of cells, preferentially mammalian tissue culture cells that have been transformed by oncogenic viruses[1]. These substances mimic antibody in that they interact to form a precipitate with biopolymers containing the 'correct' determinant sugars. Similarly, these sugars have been shown to inhibit the precipitation reaction. An interaction of concanavalin A (con A, a PHA of the jack bean) with the cell wall of *Bacillus subtilis* 168, containing glucosylated teichoic acid, has been found[2]. In contrast, the cells of a *B. subtilis* strain which produces a teichoic acid devoid of glucose residues failed to interact with the PHA. A requirement for glucosylated teichoic acid for adsorption of phage in *B. subtilis* 168 has been reported[3]. Pretreatment of glucosylated cell walls with con A results in concentration-dependent inhibition of phage ϕ25 adsorption[4].

We found that the agglutinins of *Pisum sativum* L. and *Lathyrus sativus* L. agglutinate transformable recipient cultures of *Diplococcus pneumoniae* and exhibit a concentration-dependent inhibition of the induction of competence by the competence substance in a young, not yet competent cell culture[5,6]. This indicated that PHA and the competence might bind to the same receptor sites on the surface of pneumococcal cells. We have therefore tested a series of sugars to determine at least a part of the recognition site for the competence substance on the cell wall surface. We found that only D-glucosamine and D-galactosamine inhibit the induction of competence by the competence substance in a time-dependent manner. These same sugars do not interfere with subsequent stages of the transformation process. They therefore seem to be the determinants recognised by the competence substance on the surface of inducible pneumococcal cells.

The inhibition of induction of competence by D-glucosamine and D-galactosamine, as the function of the concentration of the amino sugars used, is seen in Fig. 1. In the presence of 5 mg of the respective amino sugars, 99.3% and 97% inhibition of induction of competence was recorded, as compared with the control without the sugar (with 5% of transformants). The viability of the cells was not affected in the presence of the sugars.

A 97% and 99% inhibition of induction of competence was found when 5 mg of D-glucosamine and D-galactosamine, respectively, were first pre-incubated for 5 min with the saturating concentration of the competence substance, or when the respective sugars were added to the complex at zero time. In the case of the later additions, however, the inhibitory effect of both amino sugars decreased progressively with time, falling to zero at 21–22.5 min, when the induction of competence, in this particular case, was completed. These results (Fig. 2) demonstrate that the amino sugars interfere only at the time of the induction stage, that is the time of binding of the competence substance to the cell receptor sites. Further stages of the transformation process proceed in their presence without any disturbance.

A series of other sugars was tested, including D-glucose and D-galactose, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine, D-fructose, D-mannose D-arabinose and

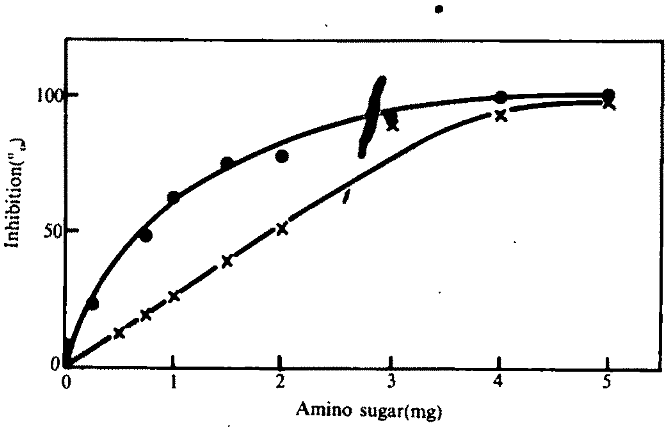

Fig. 1 The inhibition of induction of competence by the competence substance in the presence of amino sugars. D-Glucosamine HCl (●) and D-galactosamine HCl (×) were dissolved and diluted in 0.05 M phosphate buffer (*p*H 7) to obtain the required concentration, using 0.1 ml samples. Then 0.1 ml of the sample was preincubated for 5 min at 37° C in a water bath with 0.1 ml, or an aliquot amount of the competence substance at the saturating concentration. A sterile filtrate from a highly competent culture of *Diplococcus pneumoniae* of the R6-bd strain (a streptomycin-sensitive, sulphanilamide-resistant strain, isolated by Dr Tomasz), 40–60 min after the peak of competence has passed[7], was used as the source of the highly active, competence-inducing substance. One millilitre of a physiologically young, yet incompetent culture of the above strain (transferred at A_{450} of 0.08–0.1 from the pretransformation into the transformation medium[8,9] grown here for 20 min, corresponding to 3×10^6 viable units ml^{-1}, and chilled for 3 min (in ice) was then added and the cell culture suspension was induced for 20 min at 37° C in a water bath. In the control tube 0.1 ml of phosphate buffer was used instead of the solution of the tested sugar. In the control for incompetence of the recipient culture, used for induction of competence, 0.1 ml of the transformation medium was used, instead of the filtrate containing the competence substance. In a further control the filtrate with the competence substance at the saturating and subsaturating concentrations was used and the samples were completed to the given volume with the transformation medium. To all samples 0.05 ml of transforming DNA, bearing the streptomycin resistance marker (isolated from the streptomycin-resistant transformant of the R6-bd strain), at the plateau concentration level, was added and the tubes were incubated for 15 min at 37° C. The penetration of DNA was stopped by adding 0.05 ml of DNase (1 µg) in $MgSO_4$ (3 mM final concentration) to the suspension, which was incubated for further 3 min at 37° C. The tubes were then placed into ice, and after appropriate dilution of the samples, 0.1 ml aliquots were transferred on to plates with 0.2 ml sheep blood and mixed with a complete agar medium. After 2 h of incubation at 37° C the plates were overlaid with the agar medium containing 150 µg streptomycin sulphate ml^{-1}. Streptomycin-resistant transformants were scored after 16 h. 5% transformants were obtained in the control without the sugar (quoted always as percentage of added viables).

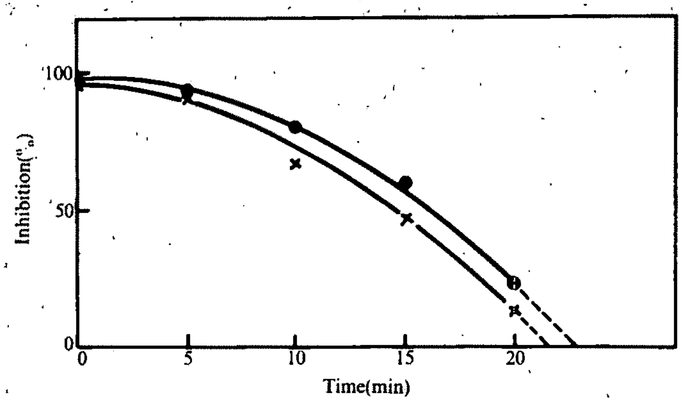

Fig. 2 The inhibition of induction of competence by the competence substance as the function of the time at which amino sugars were added to the competence substance-cell complex. 0.1 ml (5 mg) of the glucosamine (●) and of the galactosamine (×) were either preincubated for 5 min at 37° C with 0.1 ml of the saturating concentration of the competence substance, or the respective sugars were added at zero time, that is, immediately after the addition of the competence substance to the incompetent cells, and then to the 5, 10, 15 and 20 min competence substance-cell complexes, incubated at 37° C in the water bath. For induction 3×10^6 of incompetent cells (as viable units) were used. The frequency of transformants in the control was 5%.

L-fucose. Although all the sugars were tested under the same experimental conditions and at identical concentration levels, no inhibition but, rather, a stimulating effect on the induction and competence by the competence substance was found. Only with N-acetyl-D-galactosamine and with D-fructose was inhibition sometimes recorded (4–26% and 3–20% respectively), but in neither case was the degree of inhibition related to the concentration of the sugar.

The next question we posed was whether the amino sugars inhibit the induction of competence because they are the natural receptors (or determinant groups) of the competence substance to which they bind, or, alternatively, because their free amino groups compete with the competence substance for identical receptor sites at the cell surface. It has been reported that a polyamine might be an integral part of the highly basic polypeptide molecule of the competence substance[10].

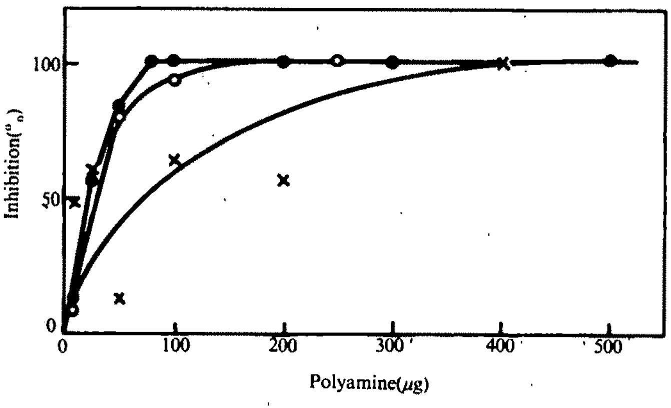

Fig. 3 The inhibition of induction of competence in the presence of protamine sulphate (●), spermine tetra-HCl (○) and histone sulphate (×). The respective substances were dissolved in the phosphate buffer and treated as described in the legend to Fig. 1. 1.3×10^6 incompetent cells were used for the induction. The frequency of transformants in the control was 1%. In the presence of 0.5 mg of protamine only 20% and in the presence of 1 mg of histone only 50% of cells (as viable units), as compared with the control without the polyamine, was found.

We investigated, therefore, whether basic substances of polyamine nature, such as protamine and spermine and the basic protein histone, would interfere with the induction process. Using the same experimental conditions as above we found that protamine and spermine completely inhibited the induction of competence, even at concentration levels of 0.08, 0.2 and 0.4 mg respectively, as can be seen in Fig. 3. But, unlike the amino sugars, they interfered not only with the induction stage, but also with further stages of the transformation process. Thus 0.1 mg of spermine and protamine produced 73% and 98% inhibition of transformation respectively when added to an already competent cell culture. All three basic substances strongly agglutinated incompetent cells, in parallel with their inhibiting effect on the induction of competence, and also produced a marked reduction in the viable cell count. It seems that these basic substances interact with the competence substance receptor sites on the cell surface, and may thus be responsible for some non-specific conformational changes which result in agglutination of the cells and a concomitant decline in the cell count.

The effect of choline, a constituent of the teichoic acid of the pneumococcal cell wall[11–13] and of its analogue, ethanolamine[14], on induction of competence was also investigated. A stimulatory effect of choline (Fig. 4*a*) and an inhibitory effect of ethanolamine (Fig. 4*b*) were recorded. When 5 mg of the ethanolamine were added to fully competent cells a 30% inhibition in the yield of transformants was found. The viable count decreased in the presence of 2 mg choline to 50%, and in the presence of ethanolamine to 33%, as compared with the viable count in the sample with the competence substance alone.

Two further experiments were performed to determine whether the amino sugars inhibit the induction of competence by binding directly to the competence substance. In the first, 1 mg and 5 mg galactosamine were pre-incubated with varying concentrations of competence substance ranging from subsaturating (0.05 ml) to hypersaturating (0.15–0.3 ml). The inhibitory effect of the amino sugar was progressively reduced as the concentration of competence substance rose.

Although this experiment suggests a direct interaction between amino sugar and competence substance, it by no means excludes a competition between them for receptor sites. A second, more direct experiment was therefore devised. The proposition put forward was as follows: if the amino sugars and the competence substance bind to identical surface receptors, these should be blocked in the presence of 5 mg D-galactosamine so that, after centrifuging, the sedimented cells should not be inducible by newly added competence substance. On the other hand, if the amino sugars bind to the competence substance, the receptors should be unoccupied so that, upon centrifugation, the amino sugar-competence substance complex should remain in the supernatant. The sedimented incompetent cells, their receptors unoccupied, should thus be inducible by newly added competence substance.

The results of the experiment were clear cut and showed that the sedimented cells, after 5 min pretreatment with D-galactosamine and saturating concentration of the competence substance could be induced by newly added competence substance to the same degree as the control without added sugar. In contrast, 100% inhibition of induction was observed in the control containing 5 mg D-galactosamine throughout the whole procedure. We infer that the inhibitory effect of the amino sugar results from its binding to, and inactivation of, the competence substance, and not to an affinity for cell receptors.

The results presented here indicate that D-glucosamine and D-galactosamine, in their non-acetylated form, are an integral part of the cell surface receptors and seem therefore to be the determinants, recognised by the competence substance, on the surface of inducible pneumococcal cells. One of the still unresolved questions to be answered is whether the free amino groups of the sugar receptors are consistent with the physiological state at which the competence substance binds to them, or whether a deacetylase has first to remove the acetyl groups from the amino groups of D-glucosamine and D-galactosamine. Only N-acetylated galactosamine and glucosamine have so far been reported to form part of the C-polysaccharide or teichoic acid of the peptidoglycan 'backbone' in pneumococcal cell wall[12,13,15] and of the teichoic acid of the cell wall in highly transformable strains of *Bacillus subtilis*[16]. On the other hand, N-non-substituted glucosamine residues in *Bacillus cereus* cell wall peptidoglycan were demonstrated and accounted for resistance of the cell walls to lysozyme[17].

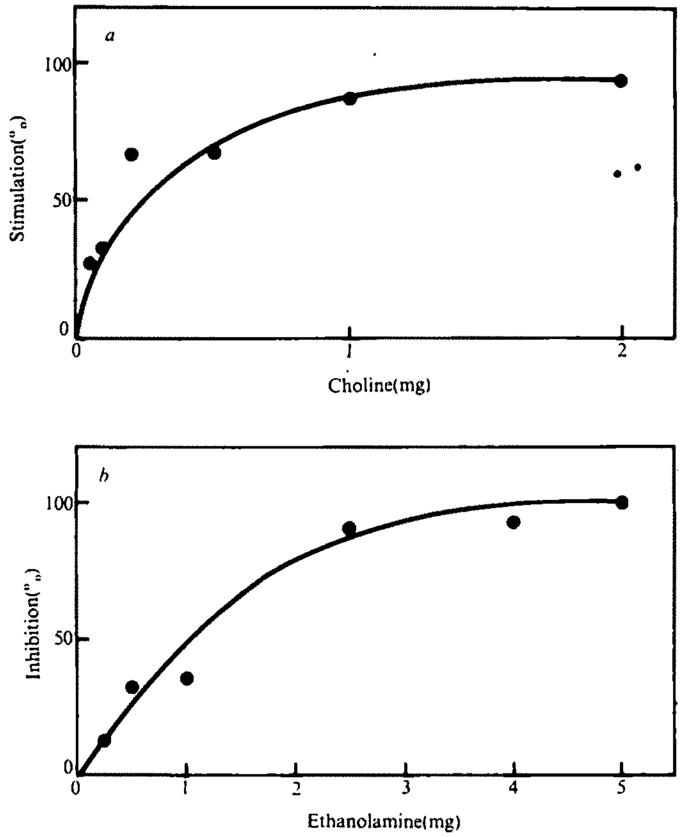

Fig. 4 The effect of choline (*a*) and of ethanolamine (*b*) on the induction of competence by the competence substance. Choline was dissolved and diluted in the phosphate buffer (*p*H 7) to obtain the required concentrations, using always 0.1 ml samples. Ethanolamine was diluted in the same buffer and the *p*H was adjusted with concentrated HCl to 7. Procedure was as in the legend to Fig. 1. 1.3×10^6 viable units ml^{-1} of the incompetent cell culture was used. The frequency of transformants in the control was 1%.

The presence of a 2-N-acetamido-4-amino-2,4,6-trideoxyhexose was also reported in the competence substance[13]. The sterical arrangement of the adjacent $—NH_2$ and —OH groups in this sugar might be identical with that of $—NH_2$ and —OH groups both in D-glucosamine and D-galactosamine. Therefore this diamino sugar can also not be excluded as a potential determinant. Investigations of this point are on the way.

The positively charged competence substance bound to its amino sugar determinants on the cell wall surface would not only impose a change in the surface charge from negative to positive, and thus enable the reversible adsorption of the negatively charged DNA, but possibly also, by introducing some conformational changes into the stereochemistry of the cell wall polymers, trigger off the autolytic enzyme activity. Thus, weak sites for the penetration of DNA to the deeper cell structures might be produced.

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Dissociation of Cell Division Stimulating Capacity for Balb/c-3T3 from the Insulin-like Activity in Human Serum

TEMIN *et al.*[1,2] have shown that insulin and serum proteins with insulin-like activity, isolated from either human or calf serum stimulate cell division in diploid populations of chick cells, while virus-transformed cells grow in the absence of these factors. An established line of rat liver cells, with a unique ability to grow in the absence of serum, releases macromolecular factors into the medium that have insulin-like activity and induce non-transformed cells to divide[3,4]; this suggests again that the factors with insulin-like activity are required to initiate cell division. Crystalline insulin stimulates DNA synthesis in BHK cells at limiting serum concentrations[5], and is a requirement for growth in Agarose of some non-transformed variants of these cells[6]. Both insulin and whole serum have a pleotropic effect on non-transformed 3T3 cells[7]; they induce RNA and protein synthesis, and prevent protein degradation. These factors are not needed for the maintenance of the pleotropic effect in virus-transformed cells[7]. In addition, insulin and serum[8] both cause a rapid decrease in the concentration of intracellular cyclic AMP in 3T3 cells, the decline of which may be a prerequisite for cell division[9,10]. We have isolated a serum fraction containing the factors that stimulate DNA synthesis and have insulin-like activity, and have found that heating separates the two activities.

Clonal Balb/c-3T3 cells were maintained on Falcon microtitre wells (area 0.3 cm^2) in Dulbecco's modification of Eagle's medium containing 4.5 g of glucose per litre and 2% calf serum (Colorado Serum Company). To study DNA synthesis, ^3H-thymidine and protein human serum fractions were added to these cultures 5–7 d after the medium was changed. Forty-eight hours later, the cells were fixed and processed for autoradiography; cell division was determined in the same cultures by determining the total cell number with the aid of a microscopic grid[11]. The incorporation of ^{14}C-U-glucose into glycogen by the muscles of the rat diaphragm *in vivo* was used as an index of insulin-like activity as described by Rafaelsen *et al.*[12]. To isolate the active fraction, whole blood was collected from healthy humans, allowed to clot and the serum was separated by centrifugation. The factors that induce DNA synthesis and exhibit insulin-like activity were extracted from the serum by ion exchange chromatography using a Dowex-50w-x8 ion exchange resin (Na^+ cycle) as described by Antoniades[13]. The adsorbed fraction was eluted with 0.1 M $NaHCO_3$ buffer (*p*H 9.5), neutralised with 0.36 N HCl and concentrated using Amicon Diaflow membranes (UM-10); the concentrate was dialysed against 0.15 N NaCl and lyophilised. Approximately 8 mg of protein were routinely obtained from 100 ml of human serum.

Figure 1 shows that crystalline insulin and the Dowex-adsorbed serum protein fraction both stimulated the incorporation of glucose into glycogen *in vivo*, by the rat diaphragm. Crystalline insulin was not responsible for the effect of the serum fraction, since none was detectable in these preparations by competitive radioimmunoassay[14]. Heating the serum fraction to 100° C for 20 min destroyed the factor(s) with insulin-like activity as determined by the incorporation of glucose into glycogen (Fig. 1). In contrast, the factor(s) that stimulate DNA synthesis in confluent cultures of Balb/c-3T3 were not destroyed during 20 min at 100° C (Fig. 2). Approximately 90 µg of protein stimulated DNA synthesis in 50% of the cells representing a ten-fold purification compared with whole serum. The factor(s) that stimulate cell division were also resistant to 100° C since the total cell number present 48 h after the addition of 200 µg of either heated or untreated serum protein was 55% greater than the control. Crystalline insulin (3–100 mU ml^{-1}) did not stimulate DNA synthesis in these confluent cultures, nor did proinsulin, protamine insulin or bovine growth hormone (Armour).

The Dowex-adsorbed serum fraction could not be used as a complete serum substitute, since freshly planted Balb/c-3T3 cells did not adhere to the plate and did not survive for 24 h, even in the presence of 5% (w/v) human serum albumin. In contrast, most of the serum proteins, that did not bind to the ion exchange resin, supported the growth of sparse populations of Balb/c-3T3, but the final saturation density of these cells was only 50% of those in an equivalent concentration of whole serum. This was not entirely unexpected since Holley[15] and others[16,17] have demon-

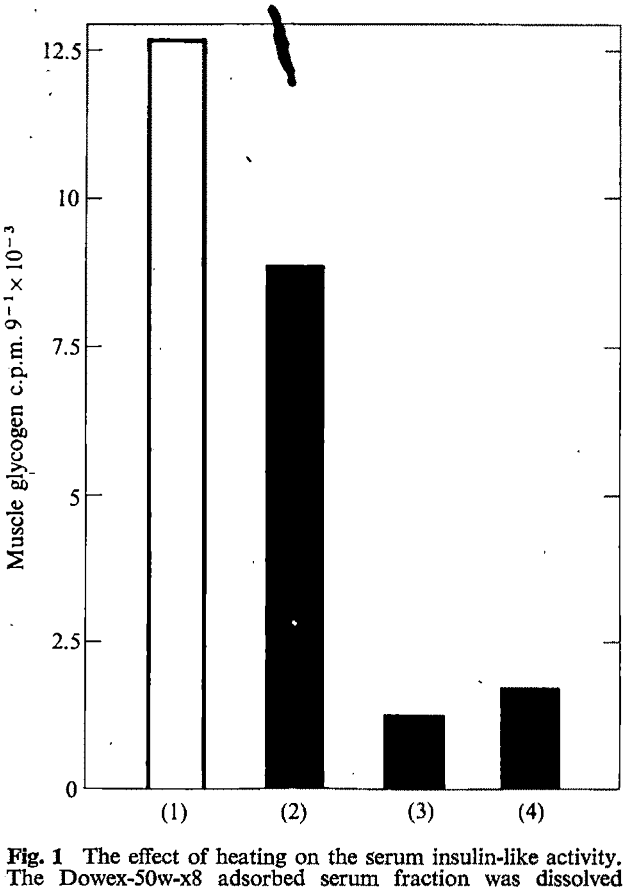

Fig. 1 The effect of heating on the serum insulin-like activity. The Dowex-50w-x8 adsorbed serum fraction was dissolved in 0.15 M NaCl (concentration 2 mg ml^{-1}) and a portion was kept at 100° C for 20 min. Insulin activity was determined by the synthesis of glycogen by the rat diaphragm using crystalline porcine insulin as a standard[12,14]. The heated or untreated serum fraction (1.6 mg of protein) or insulin was dissolved in Gey's bicarbonate buffer containing 5% (w/v) human serum albumin and together with 2 µCi of ^{14}C-U-glucose (3.3 mCi mmol^{-1}) was injected intraperitoneally (volume, 5 ml) into a non-fasted Sprague-Dawley rat (120–130 g); the buffer control did not contain the added protein fraction. Two hours later the animal was killed, its diaphragm removed, weighed, hydrolysed in 30% (w/v) KOH at 100° C, and the supernatant clarified by low speed centrifugation. The glycogen was precipitated by the addition of Na_2SO_4 to 12 mM and absolute ethanol to a final concentration of 70% (v/v); the c.p.m. represent the mean of five replicate determinations. Column (1) insulin, 1,000 µU (40 µg); column (2) adsorbed protein; column (3) adsorbed protein kept at 100° C for 20 min; column (4) buffer control.

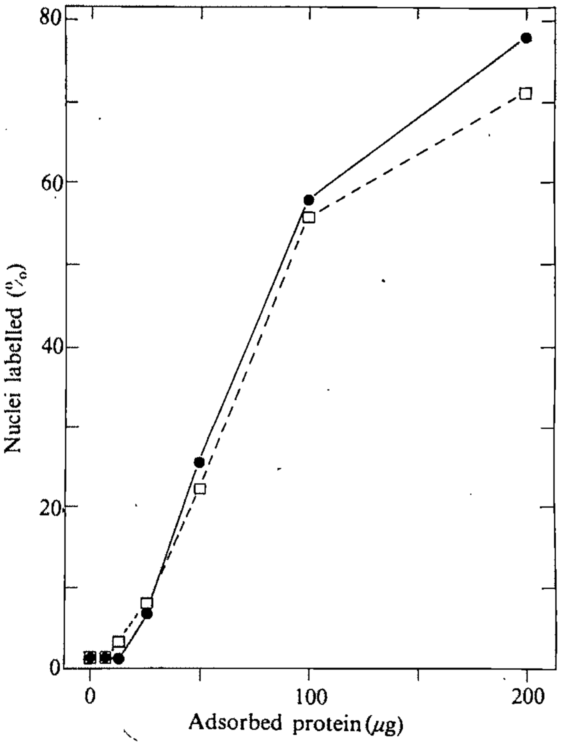

Fig. 2 The effect of serum on the DNA synthesising capacity. The Dowex-50w-x8 adsorbed serum fraction, which was heated at 100° C for 20 min as described in Fig. 1 (□) or left untreated (●), was added to confluent cultures of an early passage of Balb/c-3T3 (clone A31) in cell depleted[22] medium (total volume, 0.2 ml) containing 2% (v/v) calf serum, and ^{3}H-TdR 5 µCi ml^{-1} (6.7 Ci mmol^{-1}). Forty-eight hours later, the cells were fixed with a mixture of cold ethanol and glacial acetic acid (3 : 1 v/v), washed three times with 5% trichloroacetic acid at 4° C, once with methanol and air-dried. The microtitre wells were punched out and glued to a glass slide with the cell side up. The cells were overlaid with NTB2 photographic emulsion (Kodak), dried over $CaCl_2$ and allowed to set for 2 d; after fixation they were counterstained with haematoxylin. Two hundred cells were counted for each determination, while ~10^4 were scanned.

strated that separate serum factors are needed for the growth, survival and movement of nontransformed 3T3 cells.

These experiments demonstrate that the serum factor(s) that stimulate DNA synthesis and cell division in confluent cultures of Balb/c-2T3 are stable to 100° C and are therefore distinct from the factor(s) with insulin-like activity which are destroyed at this temperature. Pierson and Temin[2] have used cation exchange chromatography to isolate factor(s) from calf serum that stimulate cell division in nontransformed populations of cells; these proteins are sensitive to sulphydryl reduction and proteolytic degradation, and are also stable to 80° C and acid. A factor from human serum that stimulates cell division has recently been separated from the heterogenous group of factors with insulin-like activity by the use of preparative isoelectric focusing (our manuscript in preparation), demonstrating again that they are distinct molecules.

Pasten *et al.*[9] and Burger *et al.*[10] suggested that a decrease in the concentration of intracellular cyclic AMP in cultures of fibroblasts is an initiating event in cell division. A decrease in intracellular cyclic AMP, however, is not itself sufficient to stimulate cell division since crystalline insulin (80 mU ml^{-1}) rapidly lowers intracellular cyclic AMP in confluent cultures of Balb/c-3T3[8], without inducing subsequent DNA synthesis. Whole serum decreases intracellular cyclic AMP concentrations and induces subsequent cell division but contains insulin and proteins with insulin-like activity. Partially purified human serum preparations with insulin-like activity (termed somatomedin[18]) induce DNA and chondroitin sulphate synthesis in isolated costal cartilage from hypophysectomised rats. These fractions compete with insulin for membrane binding sites on isolated liver, fat and cartilage cells[19] and, like insulin, have been shown to inhibit the basal activity of adenyl cyclase in cellular membrane preparations[20,21]. Since heating destroys the factors with insulin-like activity present in human serum without affecting the factors that stimulate cell division, it may be possible for us to help clarify the role of cyclic AMP in the cellular growth cycle by learning if the serum factors that induce DNA synthesis also decrease the intracellular concentration of cyclic AMP.

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p-Fluorophenylalanine and 'Division-related Proteins'

CELLS approaching mitosis after completion of DNA synthesis are believed to synthesise certain RNA messages and to translate these in order that proteins essential for mitosis become available[1–6]. The synthesis during G_2 of 'division-related proteins' is by no means proven, however, and there is distinctly contradictory evidence in the case of HeLa cells[7]. The time spent in G_2 varies considerably between cell types and the synthesis of proteins involved in the mitotic process might be sufficiently complete by mid S phase in some cell lines while it is only accomplished by late G_2 in others. If we assume, however, that proteins specifically related to division are made in G_2, then it is of interest to know the latest time that they can be made before division, and what they do.

Some studies of the G_2 phase have involved the amino acid analogue *p*-fluorophenylalanine[2,8,9]. Experiments with bacteria[10] suggest this analogue could resolve some of the problems of division proteins, but similar experiments have not been carried out in eukaryotic cells. We analysed the effects of *p*-fluorophenylalanine on the progression of HeLa cells from G_2 into mitosis, restricting our interest to the requirements of cells to reach metaphase.

When cells incorporate an analogue such as *p*-fluorophenylalanine, the altered proteins may malfunction[10,11]. If proteins made in G_2 are essential for sending cells into division, those synthesised in the presence of *p*-fluorophenylalanine might prevent cells entering mitosis. We have carried out this experiment many times with *p*-fluorophenylalanine in HeLa cells. To avoid competition between phenylalanine and *p*-fluorophenylalanine, a precaution not adopted by others[2,8,9], cells were initially transferred to a phenylalanine-free Eagle's HeLa medium containing 10% dialysed calf serum 2 h before experimentation. This deprivation produced a slowly increasing inhibition of cell cycling only after 6–7 h. When DL-*p*-fluorophenylalanine was added at a concentration equivalent to that of phenylalanine in basal Eagle's medium (0.2 mM, 0.1 mM with respect to the L-form), entry of cells into mitosis was completely inhibited within about 15 min (Fig. 1*a*) and prophases rapidly disappeared from the cultures. Lower concentrations were less inhibitory, 0.05 mM being relatively ineffective. When competition between DL-*p*-fluorophenylala-

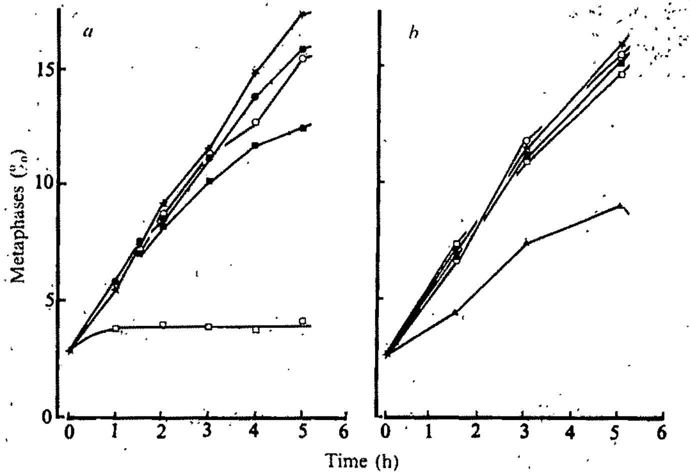

Fig. 1 *a*, Effect of *p*-fluorophenylalanine in phenylalanine-free medium on entry of cells into mitosis. Suspension cultures of HeLa S-3 cells were transferred to phenylalanine-free medium 2 h beforehand and at time 0 h, colcemid (1.5×10^{-7} M) was added. Mitotic activity is expressed as percentage metaphases from duplicate counts of 1,000 cells at each time point. Symbols, which are common to all the figures, are: ★, Control, containing 0.1 mM L-, or 0.2 mM DL-, phenylalanine; ●, phenylalanine-free medium; ○, 0.05 mM *p*-fluorophenylalanine in phenylalanine-free medium (phenylalanine medium for Figs 1*b* and 2*b*); ■, 0.1 mM *p*-fluorophenylalanine in phenylalanine-free medium (phenylalanine medium for Figs 1*b* and 2*b*); □, 0.2 mM *p*-fluorophenylalanine in phenylalanine-free medium (phenylalanine medium for Figs 1*b* and 2*b*); ▲, 0.5 mM *p*-fluorophenylalanine in phenylalanine-free medium (phenylalanine medium for Figs 1*b* and 2*b*). Where dashed lines join the points, the same symbols apply but the cultures were incubated at 40±0.2° C, not 37±0.2° C. The graph for 0.5 mM *p*-fluorophenylalanine is omitted as it falls away to the abscissa in 1–2 h. *b*, Relatively ineffective action of *p*-fluorophenylalanine at the same dose levels described in Fig. 1*a* when added in competition with 0.2 mM DL-phenylalanine in the medium.

nine and DL-phenylalanine was allowed; a 2.5 to 5.0-fold excess of the analogue was required to obtain an inhibitory effect (Fig. 1*b*).

There is evidence that *p*-fluorophenylalanine is incorporated into bacterial proteins[12,13] and that there is some suppression of protein synthesis by the analogue[14]. It has been surmised that the analogue is similarly incorporated into proteins of mammalian cells[2,8,9] although this has only been shown in rabbit haemoglobin[15], and serum, liver and muscle proteins[16]. Our results (to be published) show that incorporation also occurs into HeLa cell proteins. Thus the results of the above experiment, which agree with previous findings[9], suggest that proteins made during G_2 are rendered faulty, or their synthesis is inhibited, by *p*-fluorophenylalanine, thereby preventing entry of cells into division.

Smith and Pardee[10] found that bacteria incorporating *p*-fluorophenylalanine have an increased thermolability. Since *p*-fluorophenylalanine enters the proteins of HeLa cells, a similar thermolability might develop. Cultures were grown in the presence of DL-*p*-fluorophenylalanine at several concentrations up to the fully inhibitory level of 0.2 mM. One of each pair of cultures was kept at 37° C, the other being raised to 40° C in a Gyrotory shaker (New Brunswick Scientific Co.). Figure 2*a* shows the enhancing effect of increased temperature on the inhibitory action of low levels of *p*-fluorophenylalanine. This experiment, like the first, leads to the conclusion that 'division-related proteins' are synthesised in G_2. *p*-Fluorophenylalanine incorporated in competition with phenylalanine which were inadequate to stop entry into mitosis at 37° C also proved inhibitory at the elevated temperature (Fig. 2*b*).

Preliminary studies with inhibitors showed that at 5 µg ml^{-1}, cycloheximide and puromycin suppressed protein synthesis by 80–90% without causing significant cell death over 4–5 h. The best results were achieved, however, with a combination of 5 µg ml^{-1} cycloheximide+2 µg ml^{-1} puromycin, which suppressed protein synthesis by more than 90% as measured by pulses of ^{3}H-leucine at 4 h, without producing significant cell death (Table 1). Incorporation of ^{14}C-*p*-fluorophenylalanine was inhibited after 4 h by 90% in exactly the same manner as ^{14}C-phenylalanine and ^{3}H-leucine (Table 1). Experiments similar to those described earlier were then repeated with cultures receiving cycloheximide/puromycin treatment. The results show conclusively that inhibition of *p*-fluorophenylalanine incorporation prevents the analogue from (1) exerting its inhibitory effect on the entry of cells into mitosis (Fig. 3*a*) and (2) increasing the thermolability of proteins (Fig. 3*b*).

Table 1 Amino Acid and Analogue Incorporation into HeLa Cell Protein in the Presence and Absence of Cycloheximide and Puromycin.

Time of sample	Cycloheximide (5 µg ml^{-1})/ puromycin (2 µg ml^{-1})	Specific activity as c.p.m. per µg protein (% of control)		
		^{3}H-Leu	^{14}C-Phe	^{14}C-*p*-fPhe
2	–	38	77	81
4	–	56	171	130
2	+	5 (13%)	9 (12%)	8 (10%)
4	+	5 (9%)	13 (8%)	11 (8%)

Suspension cultures of HeLa S-3 cells were pulsed with 4,5-^{3}H-L-leucine (Radiochemical Centre, Amersham, specific activity 17 Ci mmol^{-1}) for 30 min before the time point at 1.0 µCi ml^{-1}. Cells were washed twice with ice-cold saline, treated with 4% perchloric acid and the insoluble residue washed with 80% ethanol, 100% ethanol, and ethanol/ether. After drying, the residue was taken up in 90% formic acid and aliquots taken for protein estimation and radioactive counting in a xylol/dioxane/ethanol based scintillation mixture. A Packard 2425 spectrometer was used.

1-^{14}C-DL-3-phenylalanine (Radiochemical Centre, Amersham, specific activity 59 mCi mmol^{-1}) was added at 0.04 µCi ml^{-1} to HeLa suspension cell cultures which had been prestarved of phenylalanine for 2 h. The labelled amino acid was diluted with cold DL-phenylalanine to give a final concentration of 0.2 mM. This was continuously present and samples were removed at 2 h and 4 h, and treated as described above.

3-^{14}C-DL-*p*-fluorophenylalanine-HCl (Calatomic, Los Angeles, California, specific activity 16 mCi mmol^{-1}) was given in an identical manner to ^{14}C-phenylalanine at 0.04 µCi ml^{-1} after dilution with cold DL-*p*-fluorophenylalanine to a final concentration of 0.2 mM.

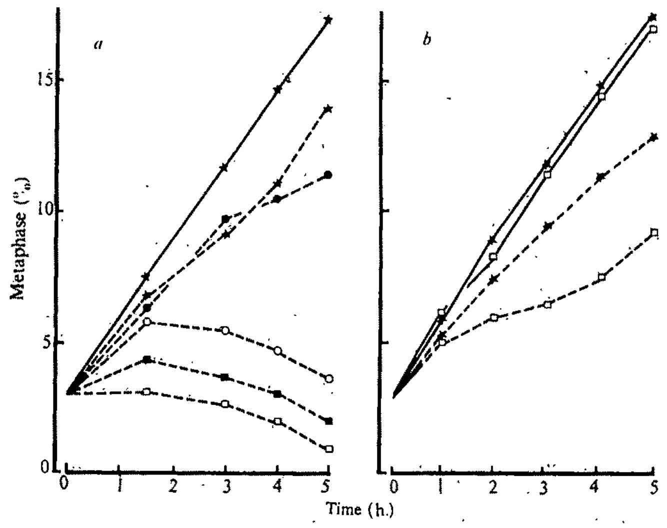

Fig. 2 *a*, Influence of raised temperature (40±0.2° C) on entry into mitosis of HeLa S-3 cells. Details as for Fig. 1*a*. *b*, As for (*a*) except, as in Fig. 1*b*, competition was allowed between 0.2 mM DL-phenylalanine and 0.2 mM DL-*p*-fluorophenylalanine.

Further inferences can be drawn from these experiments. First, the physical presence of p-fluorophenylalanine within the cell in the absence of protein synthesis does not prevent cell progression into mitosis, that is, the inhibitory action of p-fluorophenylalanine is only manifest after it has become incorporated into cellular proteins. Second, the experiments verify the observation repeatedly found in this laboratory and elsewhere[7] that inhibition of protein synthesis during G_2 does not prevent HeLa cells from reaching metaphase (Fig. 3*a*). Although cycloheximide/puromycin treatment along with raised temperature produced an accelerated loss of metaphases as pyknotic cells after 3 h, which accounts for the rapid fall-off in the lower curves of Fig. 3*b*, prophases were still appearing in these cultures at about 80% of the control rate by 5 h.

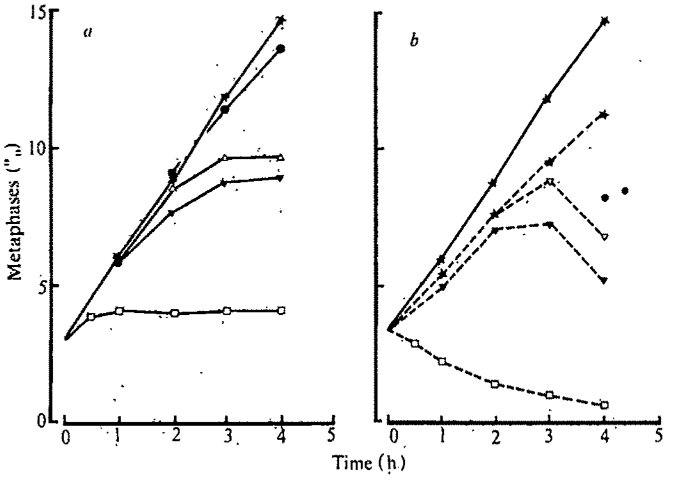

Fig. 3 *a*, Effect of inhibition of protein synthesis on the action of 0.2 mM p-fluorophenylalanine in phenylalanine-free medium. Symbols as for Fig. 1 except (△) which is cycloheximide 5 μg ml^{-1} + puromycin, 2 μg ml^{-1} added to phenylalanine-free medium, and (▼), cycloheximide/puromycin added to phenylalanine-free medium containing 0.2 mM p-fluorophenylalanine. *b*, As for Fig. 3*a* but cultures incubated at 40° C where dashed lines have been used. ▽---▽, Cycloheximide/puromycin treatment of control culture, in this case containing phenylalanine.

The relationship of p-fluorophenylalanine to the hypothetical 'division-related' proteins needs re-examination. We tentatively offer the following explanation of our results. HeLa cells entering G_2 already have sufficient amounts of the proteins required for entry into, and completion of (unpublished data), a mitotic division, as evidenced by the inability of non-lethal doses of cycloheximide and puromycin to arrest the progression of G_2 cells into mitosis (Fig. 3*a* and ref. 7). When cells are exposed to p-fluorophenylalanine, they synthesise anomalous proteins. The presence of a small, but critical amount of these anomalous proteins in the pool leads to malfunctioning of the proteins in this system as a whole and cells fail to reach mitosis. The critical level for interference by p-fluorophenylalanine-containing proteins is lowered when cells are incubated at a higher temperature because an even greater instability is imparted to the whole system under these conditions. When the synthesis of anomalous proteins is inhibited, G_2 cells progress normally into mitosis. There is no evidence that p-fluorophenylalanine *per se* prevents cells reaching division.

HeLa cells may still be preferentially incorporating p-fluorophenylalanine during G_2 into proteins involved in division although this synthesis has been shown to be superfluous to the needs of the cell for entering mitosis. We are examining the proteins synthesised in G_2 in p-fluorophenylalanine-containing medium, and the incorporation of ^{14}C-p-fluorophenylalanine into G_2 proteins. In other cell lines where the transition point from sensitivity to resistance to protein synthetic inhibitors lies closer to mitosis, the effect of p-fluorophenylalanine incorporation may have quite different consequences from those seen in HeLa cells. For this reason we are presently studying its effects on CHO cells[3].

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Reduction in Brain Dopamine following Experimental Cerebral Ischaemia

ALTHOUGH considerable information is available concerning the effects of discrete electrolytic lesions on the concentrations of the monoamine neurotransmitters in mammalian brain, little is known about the neurochemical effects of experimental vascular lesions. Such information would be useful in understanding the natural history of cerebral infarction and other neurovascular diseases; furthermore, it might suggest new therapies for treating the acute and chronic sequelae of such diseases. The observations reported here show that ligature of the middle cerebral artery in monkeys, or of the common carotid artery in gerbils, causes profound decreases in brain dopamine but not noradrenaline.

Six squirrel monkeys (*Saimuri sciura*) were subjected to transorbital ligature of the left middle cerebral artery, as described by Hodgins *et al.*[1]. Three hours later they were guillotined and the left and right cerebral hemispheres assayed for dopamine by the method of Carlsson and Waldeck[2]. Ipsilateral to the vascular lesion, the brain dopamine concentration was 0.64 ± 0.10 μg g^{-1}; in the contralateral hemisphere, this concentration was 1.13 ± 0.15 μg g^{-1} ($P<0.02$).

In developing an experimental preparation that might allow us to accumulate data from relatively large numbers of animals, we examined brain catecholamines in the Mongolian gerbil (*Meriones unquiculatus*). The blood supply to the gerbil brain is unique in lacking connecting arteries between the basilar and carotid circulations; hence, unilateral hemispheric infarction can be produced by ligaturing a common carotid artery[3]. A 50% mortality rate, equal to the infarction rate, is observed within 5 d of ligature[4]. Before death, animals may exhibit various clinical signs of infarction, including external rotation of paretic limbs when walking, hypokinesia of the contralateral side of the body, ptosis of the contralateral eyelid, and circulating behaviour, usually towards the ischaemic hemisphere and around a progressively decreasing diameter[4].

Mature mongolian gerbils (60–70 g) were anaesthetised lightly with diethylether, and the left common carotid was exposed through a ventral midline cervical incision. After the vagus nerve and the jugular vein had been dissected free, the artery was doubly ligatured with 4–0 silk sutures, and transected as previously described[3]. Groups of twelve animals were guillotined after various intervals, and their left and right cerebral hemispheres assayed for dopamine[2] and noradrenaline[5]. Brains from other animals prepared similarly were dissected into neostriatum, nucleus accumbens plus olfactory tubercle, hypothalamus, and the rest of the hemisphere, and pooled samples ($n=4$–5) were assayed for dopamine and noradrenaline. Twenty-four hours after unilateral carotid ligature, brain dopamine was reduced by 46% on the infarcted side (2.09 ± 0.39 μg g^{-1} compared with 0.97 ± 0.24 μg g^{-1}, $P<0.02$). Brain dopamine was not significantly reduced among gerbils killed 2 h after lesioning, but was depressed among animals killed after 3 h. Brain noradrenaline concentrations were not dissimilar ipsilateral and contralateral to the lesion (that is, 1.19 ± 0.076 μg g^{-1} compared with 1.04 ± 0.0[illegible]3 μg g^{-1}) among animals killed after 24 h.

Table 1 Effects of Ligaturing Left Common Carotid Artery on Regional Brain Dopamine Concentrations in Gerbils

	Sham-operated μg g^{-1}		Common carotid ligature μg g^{-1}	
	Left	Right	Left	Right
Neostriatum	1.12, 1.15	1.19, 1.18	0.69, 0.83	1.05, 1.06
Hypothalamus	1.86, 1.93	1.75, 1.83	0.80, 0.70	1.75, 1.19
Nucleus accumbens–olfactory tubercle	1.30, 1.59	1.40, 1.29	0.91, 0.71	1.58, 1.32
Rest of hemisphere	0.35, 0.37	0.34, 0.31	0.24, 0.14	0.13, 0.37

Two experiments were performed using groups of five and four animals respectively; tissues were pooled prior to assay. Animals were killed 24 h after placement of lesions.

Large brain dopamine reductions were observed in three parts of the cerebrum known to receive dopaminergic projections[6], but apparently not in the rest of the hemisphere (Table 1). The largest decreases were in the hypothalamus and the nucleus accumbens–olfactory tubercle (Table 1). Among sham-operated animals in which the common carotid artery was exposed, but not ligatured, brain dopamine was unchanged in all regions examined (Table 1).

Subtotal ischaemic necrosis of the telencephalon and diencephalon ipsilateral to the vascular lesion probably accounts for the profound decreases in dopamine concentrations within these brain regions. The area of infarction would be expected to include several dopaminergic tracts, that is, the nigro-neostriatal pathway, the tubero-infundibular pathway, and the cephalic portion of the meso-limbic pathways[3,4]. The major difference in the apparent susceptibility of noradrenergic and dopaminergic neurones to the ischaemia produced by left common carotid ligature could result from any of the following mechanisms: (1) the caudal location in the brain stem of noradrenergic cell bodies, which would continue to be nourished by the basilar artery; (2) the existence in gerbils of a single anterior cerebral artery, which would allow noradrenergic terminals in the anterior pole of the left hemisphere to be nourished by blood from the right carotid artery, and (3) differences in the susceptibility of noradrenergic and dopaminergic neurones to anoxia. The blood supply to the gerbil hypothalamus is provided by a rather large and consistent end artery derived from the anterior circulation; it is not surprising that the decrease in dopamine is most marked in this region.

Dopamine liberated from infarcted or severely ischaemic neurones could diffuse beyond synaptic clefts and interact with intracerebral blood vessels, glia, or as a false neurotransmitter with neurones that do not normally receive dopaminergic inputs. Since the inactivation of dopamine by oxidative deamination and by re-uptake into presynaptic neurones both require oxygen, the physiological effects of any dopamine released within ischaemic areas might be expected to be potentiated. Osterholm and his colleagues have suggested that, following experimental trauma to the spinal cord, noradrenaline liberated within the injured tissue causes intraspinal blood vessels to constrict, and thereby exacerbates tissue damage[7]. While the effects of dopamine on cerebral blood vessels await characterisation, this amine is known to modify vascular tone in peripheral organs[8]; hence, the possibility that brain blood flow is altered by the release of very large amounts of the amine after strokes merits exploration.

Just as the excessive release of dopamine might contribute to the acute pathophysiology of middle cerebral artery strokes, the neurological status of animals and humans who survive such strokes might also be complicated by a chronic decrease in central dopaminergic tone. That changes in brain dopamine similar to those reported here may also occur in human patients is suggested by the observation that the internal capsule of the human brain contains high concentrations of homovanillic acid[9], and thus probably dopaminergic neurones.

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Glucose Inhibition of the Glucose-sensitive Neurone in the Rat Lateral Hypothalamus

IT is generally accepted that the lateral hypothalamic area (LH) and ventromedial nucleus (VMH) have a reciprocal relationship in the control of feeding behaviour[1–3], and that some neurones in these regions are sensitive to levels of glucose in the blood[4,5] or to electro-osmotically applied glucose[6]. We have demonstrated that although about one-third of the VMH neurones increased their activity when glucose was

Table 1 Number of Neurones in which Glucose-induced Inhibition was Blocked by Ouabain or Sodium Azide

	No. of neurones tested	Blocked	Unblocked
Ouabain	7	4	3*
Sodium azide	5	4	1
	12	8	4

* Application was impossible in all three because the resistance of the ouabain pipette was more than 10^3 MΩ and this was beyond the compensation capacity of the constant current device with a maximum output voltage of ±100 V (ref. 12).

applied, there are neurones in LH which are specifically inhibited by it. These neurones are activated by direct applications of insulin, 2-deoxy-D-glucose and 3-O-methyl-D-glucose[7]. Booth[8] demonstrated that the injection of 1 μl of 5% D-glucose in the LH blocked insulin-induced eating. More recently Colin-Jones and Himsworth[9] reported that an injection of 2-deoxy-D-glucose (2DG) into the LH causes a marked increase in gastric acid secretion and attributed it to the lack of metabolisable glucose in the LH neurones. The fact that the specific glucose-sensitive cells which are inhibited by glucose exist only in LH has been highlighted in a recent report in which gastric secretion induced by the peripheral administration of 2DG in the cat was blocked by specific and discrete lesions in the lateral hypothalamus[10]. Here we report that the inhibition of LH neurones induced by direct application of glucose is the result of membrane hyperpolarisation and possibly results from the activation of the sodium pump causing an increased extrusion of internal sodium.

Twenty-four male Wistar BR46 rats, fed *ad libitum*, were anaesthetised with intramuscular urethane (1.5 g kg^{-1}). Glass microelectrodes filled with 3 M KCl, d.c. resistance 10–20 MΩ, used for recording single neurone activity, were cemented under a microscope to seven-barrelled micropipettes with α-cyanoacrylate. Intracellular recordings with extracellular glucose application were accomplished by keeping the tip of the multibarrelled pipette 10 to 30 μm higher than the tip of the recording electrode.

The multibarrelled micropipette (overall tip diameter, 1 μm) was filled with D-glucose 0.5 M per 0.15 M NaCl, sucrose 0.5 M per 0.15 M NaCl, ouabain 0.017 M, sodium azide 1.5 M, and monosodium L-glutamate 2 M. The d.c. resistance of each barrel ranged from 75 to 500 MΩ. For the application of a particular substance, a constant current of appropriate polarity and strength was passed through the barrel[11,12]. Criteria laid down by Curtis and Koizumi[13] were followed to differentiate the effect of the electric current on its own from that of the substance. The injection-recording sites in the LH were A: 5.0–4.2 (from rostral to caudal edges of the VMH); L: 1.5; H: −2.0 to −3.4 after König and Klippel's atlas[14], and confirmed histologically. The methodological details have been described earlier[11].

Out of forty-five LH neurones on which glucose was applied, ten increased the frequency of their discharges. This facilitatory effect, however, was also observed on application of Na and sucrose, showing the essentially osmosensitive nature as reported earlier by us[6,7]. Twenty neurones did not respond to glucose application. The remaining fifteen were specifically inhibited by glucose application but not by Na or sucrose application. Together with our other results, this shows that although the osmosensitive and glucose-sensitive neurones are mingled within the whole LH, the former are more common in the dorsal part and the latter more common in the ventral part of the LH. A typical intracellular record of the activity of a glucose sensitive neurone is given in Fig. 1. It is seen that the application of glucose with a constant current of 10 nA produced marked inhibition on the spontaneous spike generation accompanied by a hyperpolarisation of the membrane by about 5 mV. Moreover, change of the membrane resistance did not occur, as seen in the constant amplitude of the small hyperpolarising pulse near the start of each trace, produced by brief current applied through the bridge-balanced recording electrode. It seems, therefore, that the glucose hyperpolarisation is not due to any change in membrane permeability.

It is known that the extrusion of the intracellular sodium by sodium pump is inhibited by ouabain and by sodium azide[15–17]. Figure 2*a* shows a record in which the application of glucose at +30 nA produced a decrease in the extracellular spike discharge with a latency of 10 s and then a complete inhibition at 20 s. Application of ouabain at the dose of −50 nA led to a resumption of spike discharge at the original rate. Glucose, reapplied one min after ouabain application, did not show its specific inhibitory effect. The ouabain antagonisation of glucose-induced inhibition lasted for 2–3 min in general, after which glucose, when applied, again exhibited its characteristic inhibitory effect.

L-Glutamate was applied to relatively quiet glucose-sensitive LH neurones at first, to increase their discharge rate[18], and the effect of ouabain on the glucose-induced inhibition was tested again. As shown in Fig. 2*b* application of L-glutamate at −30 nA on the glucose-sensitive neurone increased its firing rate from 1–2 to 30–40 Hz. When glucose was applied through another barrel of the pipette at +20 nA, the spike discharge decreased after a latency of 6 s and was completely suppressed in 10 s. A superimposed application of ouabain for 20 s through the third barrel now led to the resumption of the glutamate-induced discharge. It can be noticed that the ouabain effect continued for more than 1 min after its applied current was cut off, and that the glutamate firing was resumed about 10 s after the cessation of glucose current. It was also noticed that whenever ouabain blocked the inhibitory action of glucose, it increased the spontaneous firing. These effects of ouabain were repeatedly demonstrable in four LH neurones (Table 1). The LH neurones are covered by the lamella processes of the astrocytes which are likely to impede the

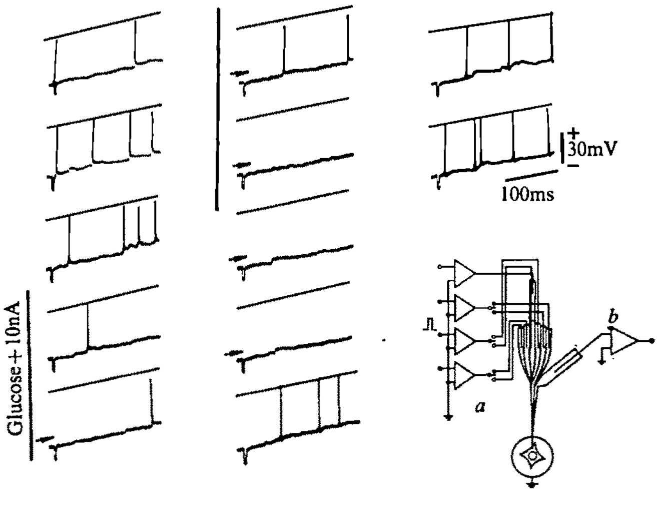

Fig. 1 Intracellular recording from a neurone in the LH depicting the inhibition of spontaneous spike discharge by extracellular glucose application. In each record the upper beam indicates the potential before the electrode penetration. Thick vertical line indicates the duration of glucose application, and the arrows indicate the level of membrane potential before glucose application. The hyperpolarisation was seen in the second record after the start of glucose application and lasted for about 3 s after its termination. The resistance of the membrane did not change because the amplitude of the 10 msec hyperpolarising pulse remained constant throughout. The time elapsed between two successive tracings was 1.3 s. The arrangement of the recording and multibarrelled micropipettes is schematically illustrated below the tracings to the right. *a*, Constant current amplifier; *b*, d.c. preamplifier.

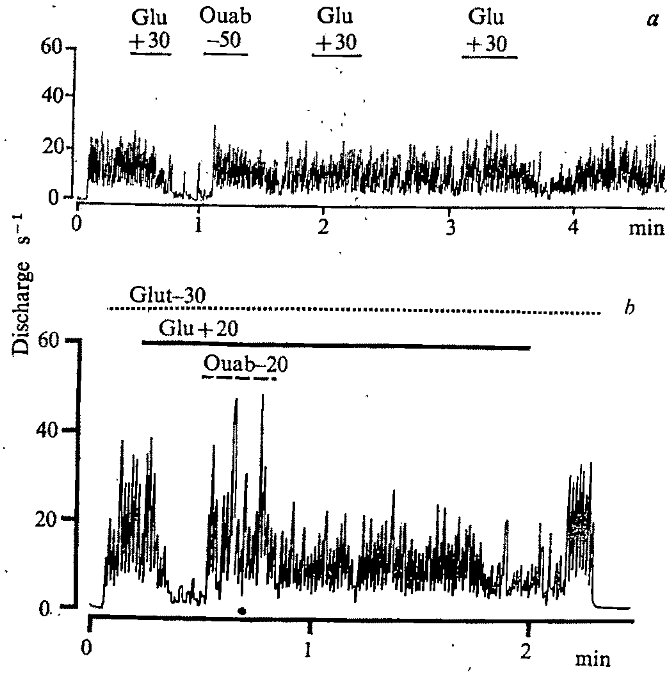

Fig. 2 Pulse counter records of the extracellular spike discharges s^{-1} of two glucose-sensitive LH neurones from two different rats. *a*, Application of glucose inhibited the spike discharge completely. Application of ouabain through another barrel of the same multibarrelled pipette restored it. Further application of glucose showed its inhibitory effect after only about 2 min ouabain application. *b*, Discharge rate of an LH glucose-sensitive neurone was enhanced by the continuous application of glutamate. Application of glucose led to its inhibition but the glucose induced inhibition was antagonised by a superimposed brief application of ouabain. The inhibitory effect of glucose was shown again only a minute or so after the termination of ouabain application. A resumption of vigorous discharge appeared with some latency when glucose application was terminated. Glut, sodium glutamate; Glu, glucose; Ouab, ouabain. The numbers along each abbreviation indicate the current strength (nA) and its direction.

diffusion of chemical injected by microtechnique[7], and the long latencies of the responses to chemical application could be due to this.

In another five glucose-sensitive neurones the effect of sodium azide was similarly tested, and it was found that glucose-induced inhibition was completely blocked in four of them (Table 1). Thus, the inhibition of these neurones by glucose seems to be caused by a membrane hyperpolarisation which is not due to a permeability change, but is due to the activation of the energy-dependent sodium pump, as it is effectively blocked by cardiac glycosides or anti-metabolites.

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Reciprocal Relationship of Alkaline Phosphatase and 5′-Nucleotidase in Human Bone

THE presence in bone of alkaline phosphatase (APase, EC 3.1.3.1.; orthophosphoric monoester phosphohydrolase) has been known for many years[1,2]. Activity increases during bone growth[1,3,4] and at the site of Paget's disease[5,6], fractures[7,8], fibrous dysplasia[9], and various bone tumours[5,7,8]. Certain authors have suggested a positive role for APase in bone mineralisation[1–3,10]. Others have stressed its importance in bone matrix formation[11]. A third view emphasises the ability of APase to hydrolyse pyrophosphates, thereby relieving their inhibition of bone mineral formation[12,13]. Dissociation between APase activity and calcification has also been demonstrated[14]. The part played by APase in bone growth and mineralisation is thus controversial.

Although 5′-nucleotidase (5Nase, EC 3.1.3.5; 5′-ribonucleotide phosphohydrolase) is more active in bone at physiological *p*H than APase[15] it has been little studied in this tissue apart from confirmation of its presence in bones and teeth[16,17] and a report of low activity on histochemical staining of regenerating bone and bone tumours induced in mice[7]. Since changes in 5Nase activity more than that of APase would be expected to alter availability of nucleic acid precursors, the relationship of the two enzymes was examined in bones with intrinsically different growth rates.

Ten femurs and five ribs were obtained from children dying within the first 3 months of life. Samples of the femoral shaft and ribs were obtained from deceased adults. All material was separated within 18 h of death and all specimens were histologically normal. Five samples of osteoblastic bone were obtained *post mortem* from the femoral shaft of patients with Paget's disease.

All samples were cleansed of flesh and marrow. The femoral epiphyses of the neonates were sliced into sections 1–2 mm thick. The remaining bones were crushed. Weighed samples were covered with 10 volumes of water saturated with chloroform and allowed to autolyse for 7 d at room temperature, after which the liquid was decanted, filtered (Whatman No. 4), and centrifuged at 5,000*g* for 15 min. The APase and 5Nase activity and, where possible, the protein content of the supernatant were measured. APase activity was estimated by assaying the hydrolysis of 0.1 mM 2′-AMP and 3′-AMP independently in 50 mM Tris HCl containing 10 mM Mg^{2+}, *p*H 7.9, at 37° C. Adenosine release was monitored by following the decrease in E_{265} which resulted from its deamination by 3.3 µg of adenosine deaminase (Boehringer) added per ml of reaction mixture. 5Nase activity was also measured using 0.1 mM 5′-AMP as substrate, APase hydrolysis of 5′-AMP being competitively inhibited by incorporating 15 mM β-glycerophosphate into the reaction mixture[18].

Protein was measured by precipitating with trichloroacetic acid, washing with organic solvents to remove lipids[19] and redissolving in 0.3 M NaOH before carrying out the Biuret reaction[20]. Results were expressed as µg protein nitrogen by reference to crystalline bovine serum albumin calibrated by Kjeldahl analysis (Armour Pharmaceutical Co., Eastbourne, Sussex). Enzyme activity as mU per mg protein nitrogen will be referred to as 'specific activity'. Statistical analysis employed the non-parametric U-test[21] to assess the significance of differences between groups, but the data were organised as mean ±s.e.m. for presentation. Only differences significant at a level of $P<0.05$ will be described.

Hydrolysis of 2′-AMP and 3′-AMP as a percentage of the rate of hydrolysis of 5′-AMP by the same preparation, corresponding to the relative proportions of APase and 5Nase in the same sample, is shown in Table 1. There is a striking difference between the femoral shaft in children and in adults. In the former, APase is ten-fold more active than 5Nase, whereas in adults the two enzymes are equally active. The femoral epiphyses, however, are more like the adult bone in respect of the ratio between the two enzymes. This ratio is higher in adult ribs than femur, and lower in neonatal ribs than femur, so although the ratio in neonatal ribs is somewhat higher than in adult ribs, the difference is not significant. Osteogenic areas of the femoral shaft from patients with Paget's disease have a higher value for this ratio than normal adult femur; in this respect they seem to revert towards the neonatal pattern.

Table 2 presents data expressing the rate of hydrolysis of the three nucleotides per mg protein nitrogen. The 5Nase content of the femoral shaft was significantly greater in adults than in children. Adult ribs had much higher 5Nase activity than neonatal ribs. The 5Nase content of femoral epiphyses was, however, higher than that of neonatal femoral shaft. The 5Nase content of ribs was higher in adults than in infants. The lowest value for 5Nase content among adults was encountered in the osteogenic areas of femur from patients with Paget's disease.

Higher values for APase were seen in the femoral shaft of children and of patients with Paget's disease than in normal adults, but adult ribs had a much higher APase content than infant ribs. Femoral epiphyses had a lower APase content than the shaft in infants.

5Nase and APase are associated with plasma membrane and cytomembrane components in liver and in most cells where their distribution has been examined[22]. The distribution of 5Nase in bone cells is unknown, but APase is concentrated in microsomal fractions in this tissue[23].

The conditions used in this work were chosen to minimise variability in release and extraction of enzymes. *n*-Butanol increased the APase content of the autolysate but inhibited 5Nase. Deoxycholate increased 5Nase activity more than that of APase and caused dissociation in their rates of release. Autolysis in water at room temperature led to progressive

Table 1 Ratio of the Rate of Hydrolysis of 2′-AMP and 3′-AMP to that of 5′-AMP in Various Categories of Adult and Neonatal Bone

Tissue	Number of specimens	2′-AMP ratio*	3′-AMP ratio*
Epiphyses of femur (children)	10	1.31±0.35 (0.24–3.57)	1.36±0.29 (0.17–2.95)
Shaft of femur (children)	9	12.24±2.46 (4.40–26.40)	11.92±2.48 (4.80–26.60)
Shaft of femur (adults)	7	0.86±0.35 (0.01–2.50)	0.96±0.36 (0.03–2.67)
Paget's disease (femur)	5	3.90±0.91 (0.88–6.44)	3.75±1.01 (0.48–6.64)
Ribs (children)	5	4.44±0.47 (3.43–6.02)	3.55±0.16 (3.24–4.00)
Ribs (adults)	19	2.63±1.25 (0.23–7.04)	2.57±1.13 (0.25–6.48)

*Mean ±s.e. with range in parentheses.

Table 2 Specific Activities of Nucleotide Hydrolysis of Various Categories of Adult and Neonatal Bone as mU per mg Protein Nitrogen

Tissue	Number of specimens	2′-AMPase*	3′-AMPase*	5′-AMPase*
Epiphyses of femur (children)	5	47.7±11.7 (14.5– 78.1)	75.3± 30.5 (14.5–187.5)	98.4± 50.3 (8.7–290.0)
Shaft of femur (children)	5	174.2± 36.5 (74.4–273.0)	162.2± 30.4 (81.0–245.5)	18.5± 1.6 (15.1– 24.5)
Shaft of femur (adults)	6	18.0± 5.5 (1.5– 33.0)	25.0± 4.4 (4.5– 34.8)	73.1± 24.1 (10.5–161.1)
Paget's disease (femur)	5	167.4± 41.1 (36.6–270.0)	159.3± 40.7 (20.0–245.0)	46.1± 8.2 (18.5– 67.5)
Ribs (children)	5	183.0± 28.6 (112.9–261.0)	158.0± 33.4 (63.1–237.5)	45.6± 10.9 (18.7– 73.3)
Ribs (adults)	14	618.4± 69.3 (261 –1,138)	513.4±113.4 (170 –1,659)	194.6± 75.8 (35 –700)

*Mean ±s.e. with range in parentheses.

increase in specific activity of both enzymes up to 7 d, and the activity ratios were relatively stable between days 5 and 7. Replicate experiments on two different portions of each of five bones showed that reproducibility of the entire procedure for specific activity and enzyme ratios was better than ±12.5%.

The physiological role of 5Nase is unknown, but may be related to regulatory control of energy metabolism[24] or cell growth[25]. The following observations suggest that depression of the APase/5Nase ratio may be a mechanism for suppressing bone growth or turnover in long bones: the ratio is lower in adult than in neonatal femur, and is raised when the femur is the site of Paget's disease. Cancellous bone, as exemplified by rib, does not seem to be governed by this mechanism since only slight differences in ratio were noted between neonatal and adult ribs. On the other hand, the specific activity of 5Nase may be a growth-regulatory phenomenon in both types of bone since lower values were found in both ribs and femurs of neonates compared with the corresponding adult bones, while a fall in this activity was noted in Paget's disease.

It is not clear why APase content of adult ribs should be higher than that of neonatal ribs whereas the APase content of the femoral shaft in neonates is much higher than that of the adult bone. This anomaly may reflect differences in the overall metabolic pattern of the two bone types, or in the function of APase in both types. The content and ratio of the two enzymes in the femoral epiphyses differ significantly from those of the shaft in neonates. This might indicate a different role for these enzymes in cell growth as distinct from calcification; or it may reflect differences in the enzyme content of functionally and morphologically different cell types in the various regions of actively growing bone.

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Interaction of Nitrite with Proteins at Gastric *p*H

THE discovery of volatile N-nitroso compounds[1] in certain foodstuffs[2] treated with nitrite and/or nitrate has prompted investigation into the conditions likely to give rise to them in food processing and also during digestion. Although other reactions of food components with nitrate and nitrite under physiological conditions are known[3], the fate of the total nitrate and nitrite added to foodstuffs as preservatives and that ingested with food has not been established. Our current investigations concern the possibility that interactions of nitrite with certain food components could result in the formation of C-nitroso compounds whose biological behaviour is largely unknown but from the few reports in the literature[4–7] are much weaker carcinogens than the corresponding N-nitroso derivatives. The nitrophenols, however, produced by oxidation of the corresponding nitrosophenol, could affect the energy-yielding processes of the cell by uncoupling oxidative phosphorylation[6].

As part of this study, we have investigated the nitrosation of bovine serum albumin under the conditions of *p*H and temperature normally recorded in the human stomach. It has been reported[8] that nitrosation of secondary amines by nitrite occurs in gastric juice, and Philpot and Small[9] have also shown that nitrosation of tyrosine residues in pepsin occurs at *p*H 3.8. This paper reports the products obtained from the interaction of nitrite with bovine serum albumin under simulated gastric conditions.

The reaction was carried out in unbuffered solutions, the *p*H being adjusted initially to 2.5 and allowed to rise during the reaction to 4.0–4.5, thus simulating normal gastric *p*H changes during digestion. The reaction has been repeated several times and the same results consistently obtained.

Bovine serum albumin (1 g) in dilute hydrochloric acid (10 ml; initial *p*H 2.5) was treated with sodium nitrite (0.069 g) in a stoppered flask, with stirring at 37° C for 2 h. The resulting yellow solution was immediately dialysed and freeze dried. Weighed amounts of the pale yellow powder were hydrolysed (1) with 6 N hydrochloric acid under nitrogen and (2) enzymatically[10]. Acidic and neutral amino acids were analysed automatically by ion-exchange chromatography and peak areas were analysed by computer.

Figure 1 shows the amino acid spectrum from a control sample of albumin, and the additional peaks produced from

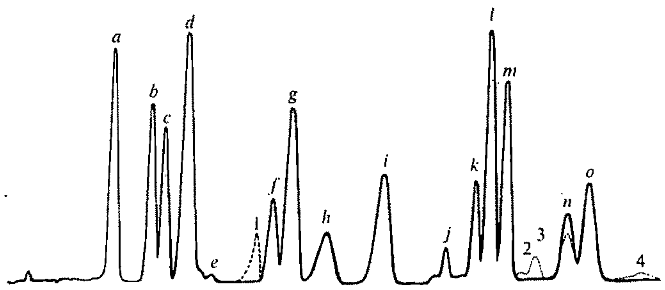

Fig. 1 Amino acids (acidic and neutral) obtained on a Beckman automatic amino acid analyser after hydrolysis of albumin (——, control; ----, after nitrosation). *a*, Aspartic acid; *b*, threonine; *c*, serine; *d*, glutamic acid; *e*, proline; *f*, glycine; *g*, alanine; *h*, cysteine; *i*, valine; *j*, methionine; *k*, isoleucine; *l*, leucine; *m*, norleucine; *n*, tyrosine; *o*, phenylalanine. 1, 6-Hydroxynorleucine; 2, unknown; 3, 3,4-dihydroxyphenylalanine; 4, 3-nitrotyrosine.

the nitrosated albumin after enzymatic hydrolysis; the amino acid spectrum produced from acid hydrolysis was similar to the latter except that peaks 1 and 2 were greatly reduced, almost to an inflection.

On the basis of their elution times by comparison with authentic compounds, peaks 3 and 4 have been identified as 3,4-dihydroxyphenylalanine (dopa) and 3-nitrotyrosine respectively. Their formation could arise by initial nitrosation of available tyrosine residues yielding 3-nitrosotyrosine, which reacts further with the decomposition products of nitrous acid. It is well known that thermal degradation of nitrous acid leads to the production of nitric acid and nitric oxide according to the equation below[11].

$$3HNO_2 \rightarrow HNO_3 + 2NO + H_2O$$

Therefore 3-nitrotyrosine could arise by nitric acid oxidation of 3-nitrosotyrosine: an analogous reaction is the nitration of phenols by dilute nitric acid which has been shown to be a two-stage mechanism involving initial nitrosation of the activated nucleus followed by nitric acid oxidation to the nitrophenol[12]. Alternatively aerial or nitrogen dioxide oxidation of the nitrosotyrosine could give rise to the same product. Whichever mechanism applies all are considered significant in the potential production of 3-nitrotyrosine in the stomach. When the nitrosated protein was hydrolysed with boiling 6 N hydrochloric acid under nitrogen without previous dialysis, residual nitrite caused extensive nitrosation of tyrosine under these more forcing conditions and following oxidation resulted in a large peak for 3-nitrotyrosine with a concomitant decrease (50%) in the tyrosine peak.

The production of dopa can be explained by reaction of 3-nitrosotyrosine with nitric oxide yielding a 3-diazonium nitrate which can be hydrolysed/photolysed to the corresponding hydroxy compound. This sequence of reactions has been demonstrated in the reaction of nitrite with *p*-cresol and the diazo derivative shown to be present by alkaline coupling with β-naphthol[13].

The presence of both peaks 3 and 4 after enzymatic hydrolysis indicates that they were produced during the reaction period rather than during the vigorous acidic hydrolysis conditions. The ratios of stable amino acids in the control and nitrosated albumins were constant within experimental error with the exception of tyrosine. In the control albumin the phenylalanine: tyrosine ratio was 1: 0.68 whereas in the nitrosated albumin this had dropped to 1: 0.60. This decrease in tyrosine residues is largely accounted for by the appearance of the dopa residues.

Peak 1 had the same elution time as an authentic sample of 6-hydroxynorleucine[14]; this probably arose by deamination of lysine ε-amino moieties. Peak 2 has not yet been identified. Loss of peak 1 during acid hydrolysis may have been due to acid-catalysed dehydration to 2-aminohex-5-enoic acid.

To summarise, 3-nitrotyrosine and dopa have been identified from the above reaction and are postulated to be degradation products of 3-nitrosotyrosine, and the ε-amino groups of lysine are deaminated to yield 6-hydroxynorleucine concurrently. This series of reactions shows that apart from nitrosamine formation there are other nitrosation reactions involving nitrite in foodstuffs, which could take place under stomach conditions and yield modified amino acids. We are currently investigating the presence of such compounds in cured meats under cooking and digestive conditions.

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Breeding System of a Sessile Animal

Chia and Rostron[1] have suggested that the planula larvae of the common intertidal anemone *Actinia equina* L. are liberated, like those of many other species of anemone, into the plankton. They then enter individuals of the same species, male or female, and are nursed in the coelenteron, eventually being ejected as juveniles of various sizes. Their reasons for suggesting this rather unusual sequence are that almost all of the eighty-five individuals they sectioned were either male or female, and that juveniles occurred impartially in both sexes.

A. equina occurs in a variety of colours, red, brownish red, brown, orange-brown, grass-green, and blue-green, plain or with stripes or dots of yellow or green on the column. The tentacles sometimes differ in colour from the column. I have kept colour varieties in the laboratory, both in mass cultures of a single colour and as isolated individuals, and scored the colours of the juveniles emitted. In general, scoring is easy even for very small young; occasionally small ones, lightly pigmented, may be so distended with water that a dull green pigment in the endoderm becomes visible and modifies the colour of the column, but this is seldom the case when they are contracted. All the juveniles emitted in my tanks that are definitely ascribable to a particular adult have the colour characteristics of that adult even to details, although less intensely pigmented when very small. Equally, all those emitted in mass cultures of a single colour variety are of that variety (Table 1). This was even the case with juveniles emitted by brown adults from Church Bay, Anglesey, although the browns are scattered among a vast preponderance of reds.

To check these results I obtained juveniles from a number of adults *in situ* in a highly variable population at Streedagh, Western Ireland (Table 2). Many individuals yielded nothing, but those that did confirmed the extremely close resemblance of juveniles to their parents (or nurses). A count of adults on the shore gave 226 red, 151 brownish red, 134 brown, 1 orange-brown, and 18 shades of green. The colours of the juveniles obtained from each adult again disagree with a hypothesis of random distribution of larvae into nurses, and with one of random cross-fertilisation.

There are several possible hypotheses to account for this resemblance. (1) It might be that each colour variety is really a separate species. However, sibling species on the shore, unless specialising in different diets, are usually strictly zoned and hardly overlap; this is certainly not the case with the colour varieties of *equina*. (2) The planula larvae might return from the plankton only to individuals of their own colour. Although Chia and Rostron[1] found some planulae

Table 1 Progeny Obtained in Aquaria

Parents		Offspring			
Locality	No.	Red	Red-brown	Brown	Green
(a) Isolated individuals					
Red					
Church Bay, Anglesey	5	30	—	—	—
Red-brown					
Plymouth	2	—	7	—	—
Streedagh, Sligo	8	—	23	—	—
Brown					
Church Bay	7	—	—	43	—
Green					
Streedagh	2	—	—	—	9
(b) Mass cultures					
Red					
Rhosneigr, Anglesey		35	—	—	—
Church Bay		29	—	—	—
Brown					
Church Bay		—	—	8	—

Table 2 Progeny Obtained at Streedagh

Parents		Offspring			
Colour	No.	Red	Red-brown	Brown	Green
Red	12	76	—	—	—
Red-brown	3	—	4	—	—
Brown	3	—	—	7	—
Green	1	—	—	—	2

in plankton samples, they had no success in trying to demonstrate their re-entry into adults. (3) The colour of a juvenile might be determined non-genetically by its nurse. Maternal effects are well known, but a combined foster-mother and foster-father effect would be unique. Also phenotypic maternal effects are not usually persistent, if only affecting colour, for a long period in the juvenile's life. (4) There may be considerable self-fertilisation together with genetic determination of the colours. Chia and Rostron[1] found one hermaphrodite (with both sexes in the same gonad), fifty-four females and thirty males. They noted that only a few mesenteries in an individual are carrying ripe gonads at one time. In so long-lived an animal, without the well-known synchronised outpouring of gametes characteristic of many sessile marine forms, a considerable amount of self-fertilisation could take place during occasional periods when an individual was effectively hermaphroditic, and thus stock itself with juveniles for many months. If the colours are genetically determined, only this hypothesis seems to explain the findings, and work is in progress to test it. The further problem immediately arises of how as many different inbred lines as must occur at Streedagh continue to co-exist on the shore without obvious ecological differentiation.

The colours are known to change if individuals are fed on a diet lacking in carotenoid; all my specimens have been fed on a carotenoid-rich diet and have shown no change over periods, for some of them, of many months. Deprived and decoloured individuals produce young with the same lack of colour[2]. Deprived adults revert to their original colours on an adequate diet[3]. Sir J. G. Dalyell's pioneering studies[4] gave much information on the breeding of *equina*. He mentioned explicitly 708 juveniles from five liver-red adults (and he probably saw over a thousand), all of which at emission were red, pink, or, in a few cases, nearly white. Of an unspecified number produced by the bright vermilion variety, all were bright red; one turned green at 5 months and produced more than 103 progeny, all green like itself. From the constancy of colour in all the others (except one very aged individual), it is unlikely that this one changed by deprivation. This is the only instance in the literature of such a change and must surely be genetic, either a mutation or a stray cross-fertilisation by a sperm from another individual also carrying green as a recessive. Dalyell[4] saw only liver-reds and vermilions in his populations in the Firth of Forth. Chia and Rostron[1] give no information on the colours of their specimens, but do mention that all the planulae were pinkish or orange. It is possible that red varieties form nearly the whole population on the east coast of Great Britain, and the problem of inheritance of colour varieties was not brought to their attention.

If the hypothesis of a high degree of self-fertilisation is correct, it will give an example of convergent similarity in breeding system and ecogenetics between a sessile animal and many plants. Sessile animals should provide interesting material for such a comparison.

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Blue-Green Bacteria synthesise L-Tyrosine by the Pretyrosine Pathway

THE similarity of major biochemical pathways throughout the animal and plant kingdoms[1] presumably reflects the ancient formation of the pathways in a common ancestral counterpart of modern cell types. The pathway previously known for L-tyrosine biosynthesis in both prokaryotic and eukaryotic microorganisms (Fig. 1*a*) involves a dehydrogenation-decarboxylation conversion of prephenate to 4-hydroxyphenylpyruvate followed by transamination in the presence of a suitable amino donor. Our data (Fig. 1*b*) demonstrate a reversed order of these two reactions in several species of blue-green algae (more appropriately designated bacteria (order Chroococales[2])).

Comparing the regulation of aromatic amino acid synthesis in *Agmenellum quadruplicatum*[3] and *Anacystis nidulans* (Kratz–Myers strain), we found that most of the key enzymes were stable. Anthranilate synthetase, 3-deoxy-D-arabinoheptulosonate 7-phosphate (DAHP) synthetase, prephenate dehydratase, chorismate mutase and an aromatic transaminase all had relatively high specific activities. In contrast, activity for prephenate dehydrogenase, the enzyme expected to catalyse the first reaction unique to L-tyrosine biosynthesis, was not detected. Enzymes able to catalyse the two reactions shown in Fig. 1*b* have been partially purified from crude, cell-free extracts. The amino acid intermediate, β-(1-carboxy-4-hydroxy-2,5-cyclohexadien-1-yl) alanine, hereafter denoted 'pretyrosine', has been prepared enzymatically (legend, Fig. 1).

A. quadruplicatum, the most intensively studied of the species in our collection, provided most of the evidence for the pretyrosine pathway of L-tyrosine synthesis. We assayed for prephenate dehydrogenase in crude and partially purified enzyme preparations (Sephadex G-200 eluate fractions) at temperatures between 30° and 45° C (2° C intervals) over a

pH range of 6.5–9.0 (0.5 pH unit intervals). Wide variations in ionic strength, the presence of amino acids, or the use of stabilising compounds (such as glycerol or β-mercaptoethanol) revealed no detectable activity. Although described prephenate dehydrogenase activities are NAD$^+$-dependent, we also tested for a possible NADP$^+$-dependent activity with negative results.

A unique route for L-tyrosine synthesis was suggested, however, by a transaminase enzyme reactive with prephenate as an amino acceptor. The enzyme was partially purified by Sephadex G-200 gel filtration giving a single eluate peak of activity that could utilise prephenate efficiently. Amino donor specificities were characterised by thin-layer chromatography. Transamination of prephenate was confirmed by the appearance of a new ninhydrin-positive spot after thin-layer chromatography. L-Leucine, L-isoleucine and L-phenylalanine were the best amino donors for transamination. Table 1 shows that when L-phenylalanine was the amino donor, prephenate was one of the best substrate reactants.

Table 1 Keto Acid Specificity of Prephenate Transaminase

Keto acid substrate	Specific activity
α-Ketoglutarate	10.4
Prephenate	8.5
α-Keto-β-methylvalerate	5.5
α-Ketoisovalerate	3.1
α-Ketobutyrate	0.9
Pyruvate	0.4
Oxalacetate	0

The reaction mixtures consisted of 0.1 ml of 40 mM L-phenylalanine (amino donor), 0.1 ml of 10 mM keto acid, 0.05 ml of 1 mM PLP, and 0.05 ml of partially purified enzyme (132 µg protein). The reaction was terminated after 20 min of incubation at 45° C by addition of 0.9 ml of 2.67 N NaOH, and the absorbance of phenylpyruvate formed was measured at 320 nm. Specific activity is expressed as nmol min^{-1} mg^{-1}. Prephenate was prepared by the method of Gibson[4].

Thin-layer chromatography of the transamination reaction between L-phenylalanine and prephenate gave two ninhydrin-positive spots; one had the same R_F as L-phenylalanine (the standard), and the other was presumably the transamination product of prephenate, that is, pretyrosine. Prephenate is quantitatively converted to phenylpyruvate in 10 min at acid pH (0.1 N HCl) by a well documented dehydration-decarboxylation reaction[5]. Likewise, if our proposed structure for pretyrosine (Fig. 1) is correct, it would be converted to L-phenylalanine at acid pH. The product of acid-treated pretyrosine migrated at an R_F identical to that of L-phenylalanine after thin-layer chromatography. To confirm this identity, a reaction mixture of prephenate, L-leucine, and partially purified transaminase was incubated for 60 min at 45° C. The protein was discarded after acid treatment, and the supernatant was analysed using a Beckman model 120-C amino acid analyser. Apart from the L-leucine initially present as amino donor, only L-phenylalanine was detected.

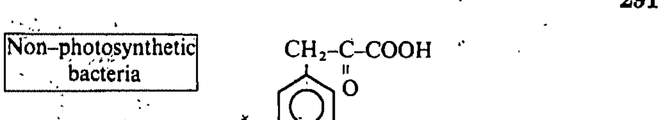

Fig. 1 Biosynthesis of L-tyrosine in prokaryotes. *a*, The 4-hydroxyphenylpyruvate pathway; *b*, the pretyrosine pathway. Abbreviations: PLP, pyridoxal-5′-phosphate; NAD$^+$, nicotinamide adenine dinucleotide. Enzyme denotations: (1) prephenate dehydrogenase; (2) 4-hydroxyphenylpyruvate transaminase; (3) prephenate transaminase; and (4) pretyrosine dehydrogenase. Pretyrosine for use as substrate was prepared as follows. A reaction mixture of 7.2 mM prephenate, 20 mM L-leucine, partially purified transaminase from *A. quadruplicatum* and 10 mM Tris buffer, pH 8.5, in a volume of 20 ml was incubated for 90 min at 45° C. After removal of protein by passage through an Amicon Diaflow cell (PM 10 filter) the reaction mixture was applied to a Dowex 21K column in the Cl$^-$ form which had been equilibrated with 10 mM ammonium formate, pH 7.5. The column was washed with approximately 30 ml of the 10 mM ammonium formate until no L-leucine could be detected in the effluent by thin-layer chromatography. Pretyrosine was eluted with 0.3 M ammonium formate, pH 7.5, and prephenate remained bound to the column. Fractions containing pretyrosine (located by thin-layer chromatography) were pooled. Further purification attempts led to significant recovery losses owing to the chemical lability of pretyrosine. Because it was stable when stored frozen in 0.3 M ammonium formate, it was used in this form. The concentration of pretyrosine was approximately 5 mM (determined by acid conversion to L-phenylalanine followed by fluorometric analysis of L-phenylalanine concentration (*Sigma tech. Bulletin* No. 60F)).

The second reaction of L-tyrosine biosynthesis in *A. quadruplicatum* is the dehydrogenation of pretyrosine. An active enzyme able to catalyse the reaction was isolated by Sephadex G-200 gel filtration. (The preparation of pretyrosine is described in the legend to Fig. 1.) The reaction was confirmed using an assay mixture of 0.1 ml of 5 mM NAD$^+$, 0.1 ml of partially purified pretyrosine dehydrogenase and 0.1 ml of 5 mM pretyrosine. The following confirmatory determinations were made. (i) Disappearance of pretyrosine: after 90 min of reaction at 37° C, 10 µl was spotted on a cellulose plate (as described above); a pretyrosine spot was barely detectable. (ii) The continuous formation of NADH: NADH was measured fluorometrically at 37° C with an Aminco Bowman spectrophotofluorometer using an excitation wavelength of 340 nm and an emission wavelength of 460 nm. The initial rate of formation was rapid when the above reaction mixture was used (specific activity, 40.9 nmol min^{-1} mg^{-1}). (iii) Appearance of L-tyrosine: after 90 min at 37° C fluorometric assay[6] of the reaction mixture indicated 100% conversion of pretyrosine to L-tyrosine, while amino acid analysis verified that L-tyrosine was the only amino acid detectable.

Although major biochemical pathways in microorganisms commonly involve an identical sequence of steps, a diversity of regulatory patterns has evolved[7]. Thus in *A. quadruplicatum* with its different pathway, novel aspects of regulation are not unexpected. The activity of prephenate dehydratase (Fig. 2) is controlled by both L-phenylalanine and L-tyrosine. L-Phenylalanine (1 mM) causes 90% inhibition, while L-tyrosine at the same concentration stimulates activity about two-fold. The results of our *in vitro* transaminase enzymology indicate that a single transaminase catalyses both the phenylpyruvate transaminase reaction (denoted (2) in Fig. 2) and the prephenate transaminase (2′) reaction.

The regulatory pattern depicted in Fig. 2 can be summarised as follows. In panel (*a*) where L-tyrosine is present in excess, prephenate dehydratase possesses an advantage in its competition with prephenate transaminase for prephenate. This, in conjunction with the increased supply of phenylpyruvate, leads the transaminase to function primarily as phenylpyruvate transaminase. Hence, in the presence of L-tyrosine, metabolite flow is pivoted toward L-phenylalanine synthesis. In Fig. 2*b* L-phenylalanine is assumed to be present in excess, and the regulatory features that tend to direct metabolite flow toward L-tyrosine are illustrated. Prephenate is shunted toward L-tyrosine synthesis owing to (i) feedback inhibition of prephenate dehydratase, and (ii) the effectiveness of L-phenylalanine as an amino donor in the prephenate transaminase reaction. These regulatory relationships appear well suited to maintaining balanced intracellular levels of L-tyrosine and L-phenylalanine.

In addition to *A. quadruplicatum* and *A. nidulans*, pretyrosine dehydrogenase activity (but no prephenate dehydrogenase activity) was verified in crude extracts prepared from four other species (obtained from L. O. Ingram); *Coccochloris elabens* strain 17A[3] (unicellular, marine); *Nostoc muscorum* strain Mac[9] (filamentous, freshwater); *Lyngbya lagerheimii* strain Mont[10] (filamentous, marine); and *Oscillatoria* sp. strain SFO (filamentous, marine; isolated by Ellen Brandt). Partial purification will be required to confirm the expected presence of prephenate transaminase in these species.

Blue-green bacteria and other prokaryotes are thought to have diverged from a common ancestor 3–4×10^9 yr ago[11]. If further comparative biochemistry is consistent with our findings, it would provide significant data that might help to clarify the evolutionary sequence. It is commonly speculated that the mitochondrial and chloroplast organelles of eukaryotes are endosymbiotic descendants of ancient prokaryotes. A logical corollary of this hypothesis is that the ancient origin of mitochondria and chloroplasts can be identified with non-photosynthetic and blue-green prokaryotes, respectively. The possibility that blue-green bacteria can be clearly distinguished from other prokaryotes by L-tyrosine pathway enzymology should provide a direct test of this evolutionary hypothesis.

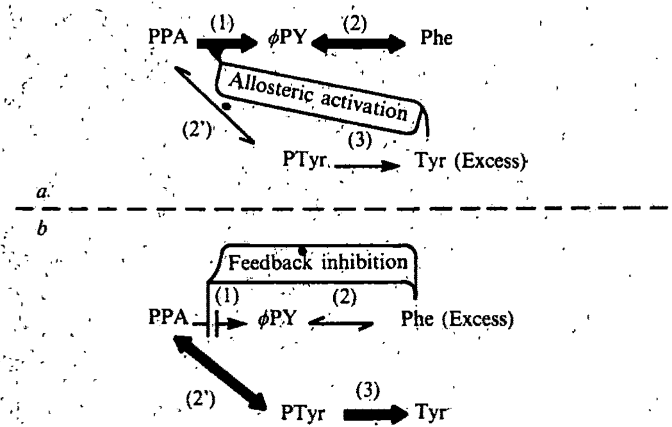

Fig. 2 Regulation of L-tyrosine and L-phenylalanine biosynthesis in *A. quadruplicatum*. *a*, L-Tyrosine is present in excess and L-phenylalanine is limiting. *b*, L-Phenylalanine is present in excess and L-tyrosine is limiting. Heavy arrows denote the metabolite shift toward L-phenylalanine synthesis in the presence of excess L-tyrosine (*a*) and toward L-tyrosine in the presence of excess L-phenylalanine (*b*). (1) Prephenate dehydratase; (2) phenylpyruvate transaminase; (2') prephenate transaminase; (3) pretyrosine dehydrogenase. Prephenate dehydratase activity was assayed as described by Jensen[8], except that 50 mM Tris buffer, *p*H 7.8, was used.

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Electron Microscopic Visualisation of Chromosomes banded with Trypsin

CHROMOSOME banding methods have been widely used to identify normal and rearranged chromosomes[1-11] and to probe the structural and biochemical organisation of chromosomes[11-13]. The methods most commonly used include Q[1,2], G[3-6], C[7] and R banding[8], each producing characteristic bands on metaphase chromosomes as observed by light microscopy. Attempts to observe banding at an ultrastructural level have not been particularly successful[12,14,15], but since such studies could provide more detailed maps of chromosomes and help to clarify the structural nature of the bands, further attempts were made in my laboratory to develop a reliable and simple method for observing chromosome bands at the electron microscope level, using trypsin as the banding agent[5].

Log phase Chinese hamster Don cells (American Type Culture Collection, Rockville, Maryland) were accumulated at metaphase by 4 h of incubation with colcemid (0.05 μg ml^{-1}) in McCoy's medium 5A+10% foetal calf serum. Metaphase cells were then dislodged into the medium by shaking, collected by centrifugation, treated with 0.075 M KCl for 15 min at 37° C, and fixed for 60 min with three changes of fixative (one part glacial acetic acid : three parts methanol). Formvar films (0.5% formvar in ethylene dichloride) were made on glass microscope slides and then coated with carbon. Each coated slide was slowly lowered into a shallow trough of water so that the film detached and floated on the surface of the water. A piece of wire gauze was placed on the bottom of the trough, and 75 mesh electron microscope grids were arranged on top of the gauze. Just before use, the fixed cells were suspended in a mixture of one part glacial acetic acid and six parts methanol, that is, the concentration of acetic acid in the original fixative was reduced. A drop of this cell suspension was placed on top of the floating film and air-dried. The wire gauze, holding the grids, was then raised out of the water so that the film, supporting the cells, was spread smoothly over the grids. After drying at room temperature, individual coated grids were removed from the gauze and checked with a phase contrast microscope to ensure that well-spread chromosome preparations were present.

When fixed cells are spread on formvar, or formvar-carbon films attached to glass slides (glass substrate), the acetic acid in the fixative disintegrates the film. This problem has been circumvented by using water as a substrate for the film, since water protects the formvar from the fixative in some unknown manner. The reduction in the acid content of the fixative just before spreading the cells seems to safeguard the film further. These precautions render the film impervious to the fixative, and high quality metaphase preparations can be made.

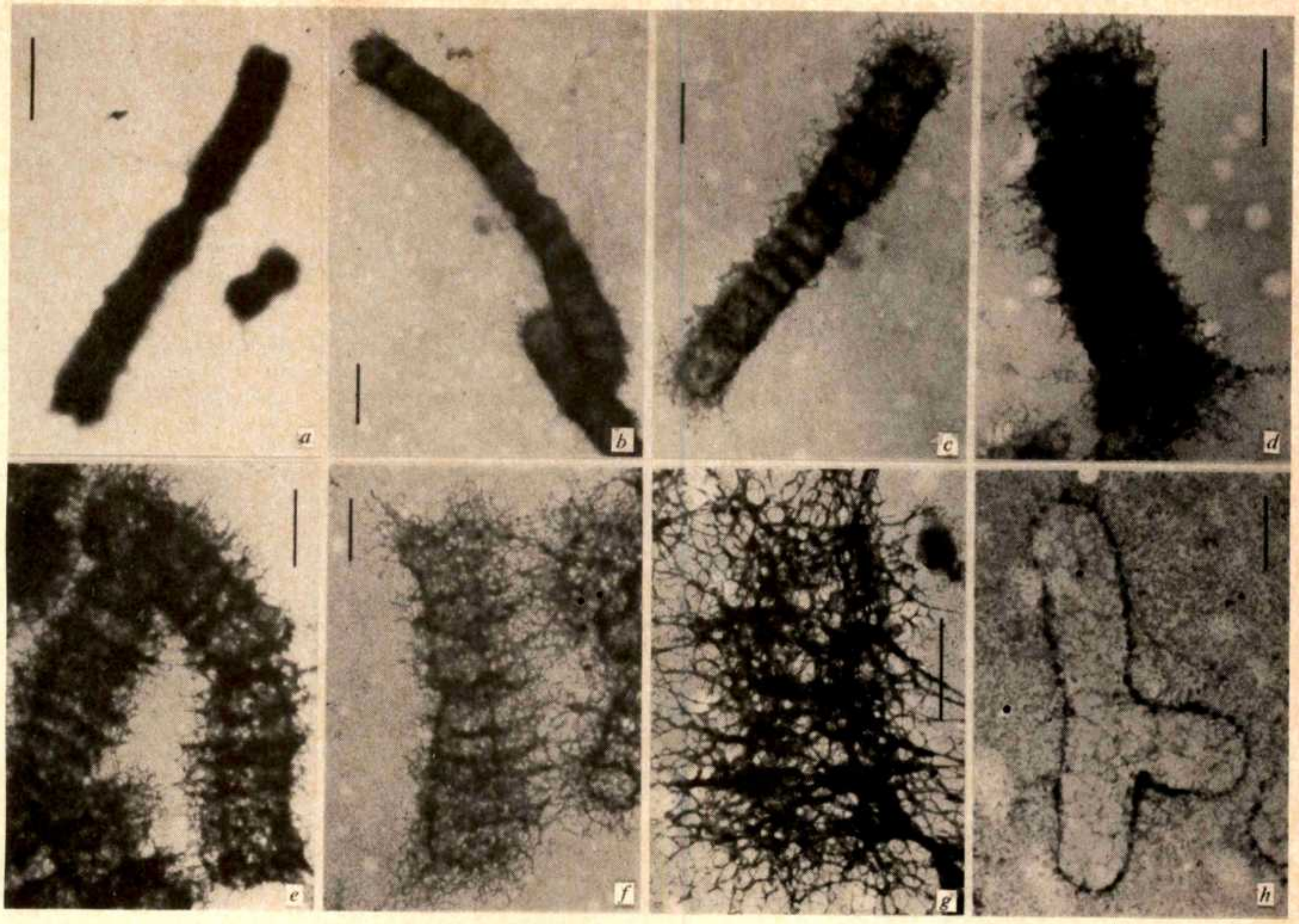

Fig. 1 The bars represent 2 μm. *a*, Untreated (control) Chinese hamster chromosomes, stained with uranyl acetate. *b*, Banded chromosome, treated with trypsin for 1.5 min, unstained. *c* and *d*, Chromosomes treated with trypsin for 2 min (*c*) and 1.25 min (*d*), unstained. *e* to *g*, Chromosomes treated with trypsin for 1.5 min (*e*) and 2 min (*f* and *g*); *f* is unstained; *e* and *g* are stained with uranyl acetate. *h*, Two overlapping chromosome 'ghosts'. Trypsin treatment lasted 10 min; Giemsa stain. Residual chromatin fibres can be seen.

For G banding, grids were floated face down on 0.1% trypsin (Difco), made in Ca^{2+}-free and Mg^{2+}-free balanced salt solution, for 1 to 10 min. Each grid was rinsed in two changes of 70% ethanol and two of 100% ethanol, and air dried. The extent of banding was monitored by placing the grid on a glass slide and examining by phase contrast microscopy.

This electron microscopy method of chromosome preparation and G banding is the same as for light microscopy, and allows comparisons of the same chromosome to be made at both levels of resolution. For electron microscopy, trypsin-treated or untreated (control) chromosome preparations were: (1) left unstained, (2) stained with Giemsa for 5 min, or (3) stained with 2% uranyl acetate for 10 min, dehydrated through a graded series of ethanol washes and rinsed in amyl acetate for 20 min before air-drying. Specimens were examined with a Philips 200 electron microscope, operating at 60 kV.

Untreated chromosomes stained with uranyl acetate or Giemsa appeared to be very electron-dense structures at the ultrastructural level (Fig. 1*a*). The chromosome morphology was well preserved. Some of the preparations stained with uranyl acetate suggested a hint of lateral striations along the chromosomes, but the Giemsa-stained chromosomes were particularly dense in appearance, and showed no fine detail. Chromatin fibres, like those seen in whole mount preparations[16,17], were not apparent in untreated chromosomes, suggesting that these fibres had all become melded together, presumably as a result of the fixative. This, however, proved not to be the case when the trypsin-treated chromosomes were examined.

Bands were readily apparent in trypsin-treated chromosomes regardless of whether the chromosomes were stained with uranyl acetate, or Giemsa, or were left unstained. At low magnifications, complete chromosome complements were observed (Fig. 2). Depending on the extent of trypsin digestion, the chromosomes exhibited different degrees of disruption. In mildly treated chromosomes, bands appeared as electron dense areas, while interband regions were much less dense (Fig. 1*b*). Preliminary results suggest that the electron dense bands correspond to the G bands observed by light microscopy. Some of the banded chromosomes showed little or no dispersion of the chromatin fibres (Fig. 1*b*); however; in many others, chromatin fibres appeared to spew out of the chromosome, particularly from interband regions (Fig. 1*c* and *d*). More severely treated chromosomes showed an extensive dispersion of chromatin fibres, apparently affecting both bands and interbands (Fig. 1*e* to *g*); however, even in severely distorted chromosomes, the bands could still be identified as such (Fig. 1*f* and *g*), suggesting that the packed or folded chromatin in the band regions was resistant to the dispersive effect of the trypsin. Completely overdigested chromosomes resembled ghosts; however, a few remaining chromatin fibres could still be identified (Fig. 1*h*).

In some trypsinised preparations, chromatin fibres could be seen very clearly (Fig. 1*g*), and measurements indicated that many were comparable in diameter (about 250 Å) to those found by whole mount electron microscopy[16,17]. The methods of chromosome preparation (hypotonic, fixation) and trypsinisation do not seem adversely to affect the chromatin fibres, at least as far as dimensions are concerned.

These results indicate that fine structural studies of G banded chromosomes are now feasible. There may be many previously unidentifiable structural details in chromosomes which could be particularly useful in cytogenetic studies, and the high degree of resolution offered by this method should permit the construction of more detailed chromosome maps which could be of considerable value in pinpointing the precise location of structural rearrangements.

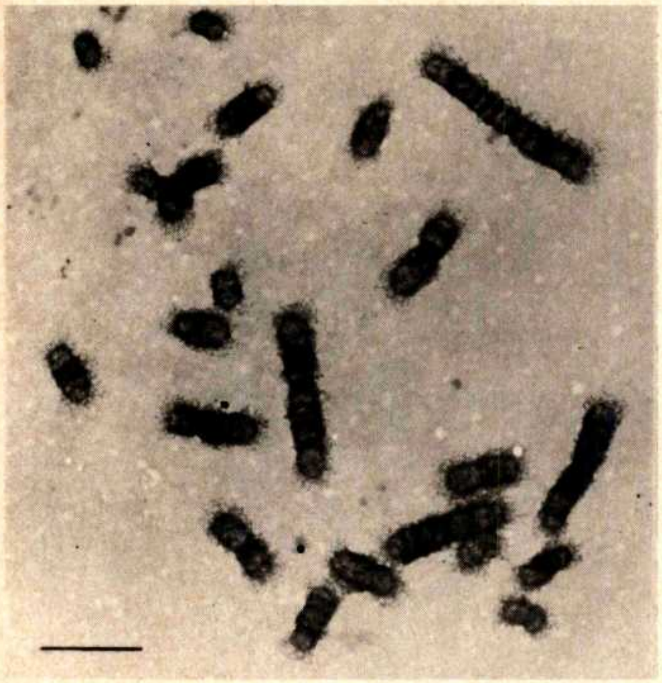

Fig. 2 Electron micrograph of a complete Chinese hamster chromosome complement, treated with trypsin for 2 min. Unstained preparation; the bar represents 5 μm.

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What Abrogates Heart Transplant Rejection in Immunological Enhancement?

IMMUNOLOGICAL enhancement is historically the oldest method for impeding graft rejection. Such an outcome resulting from the injection of antiserum directed against a transplant seemed paradoxical, and the mechanism has been under discussion all through the years of study of antigens as putative tolerogens to the present time when immunological enhancement is the best available method for donor-specific immunosuppression in animal models[1]. The shortcomings of non-specific immunosuppression at present used in clinical transplantation are so great that an interpretation of the mechanism of immunological enhancement is important, in order to find how the method can best be adapted for clinical use.

That antibodies against gene products of the major histocompatibility genetic complex (MHC) are responsible for mediating passive enhancement seems compelling from studies in which congenic strains of mice have been used. This was interpreted in the sense that in mice, H-2 antigens, the much studied serological markers for the mouse MHC, and their antibodies, played the key role. Any such conclusions would apply equally to HL-A in man, Ag-B (H-1) in the rat, and so on, because these are genetically homologous chromosomal regions.

Enquiry into the mechanisms of passive enhancement has mainly followed two courses: studies of the class of anti-

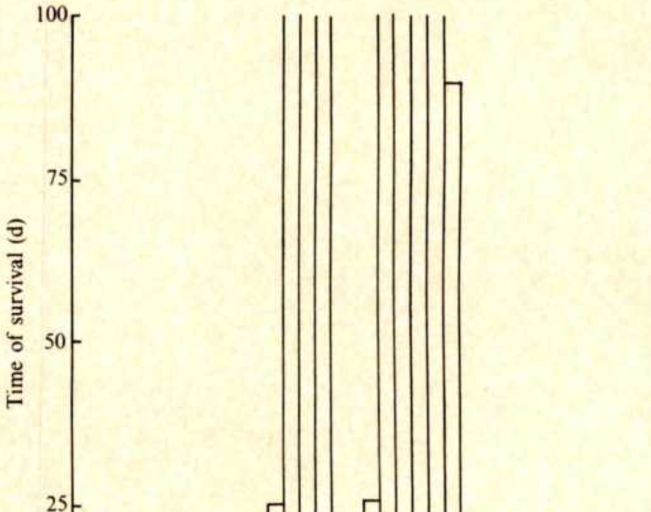

Fig. 1 Survival of rat auxiliary heart transplants from (*A/gus* × *Wag*) F_1 to *A/gus*. Series *a*, controls injected with normal rat serum, 1 ml (intravenous) on day of grafting and days 1, 3, 5, 7, 9 thereafter. Series *b*, injected (the same dose schedule) with enhancing alloantiserum; series *c*, treated with the same serum but fully absorbed with *Wag* erythrocytes; series *d*, the same serum fully absorbed with *Wag* erythrocytes and subsequently absorbed with *Wag* spleen/thymus cells.

body involved[2], and of the mechanisms by which they act (peripheral, afferent or central blocks). Recent work with enhanced rat kidney allografts shows some unbound antigen in the grafted tissue[3] and will focus attention on the favoured 'central block' hypothesis. This is based on the idea that in passive enhancement, antibody binds to transplantation antigens from the graft and the complex blocks the lymphocyte recognition site because this site is specifically related conformationally to that antigen. There is much supporting evidence for this[4] but unfortunately the nature of the T lymphocyte recognition site is not known. Whether it is an IgX or not, Ir (immune responsiveness) genes located in the MHC chromosomal region are involved with antigen recognition in some as yet undefined way. Different biological roles have been attributed to various segments of the MHC (ref. 5) and it has been suggested that the MHC associated Ir gene product might be the T cell recognition site[6].

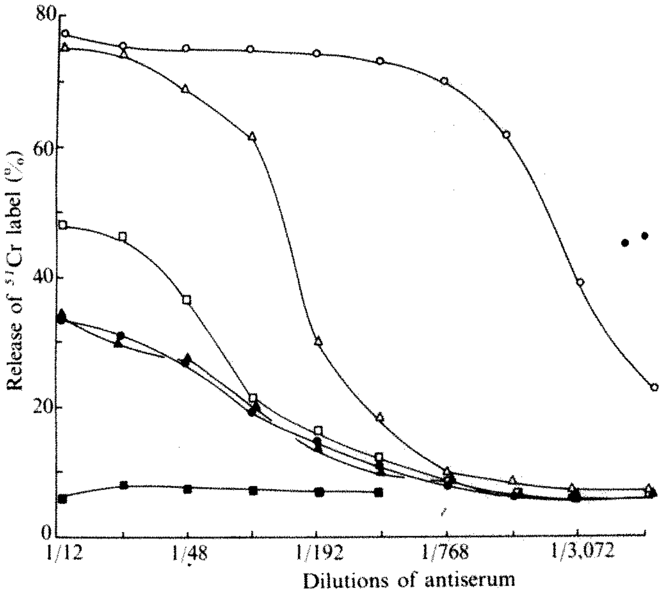

Fig. 2 Absorption of *A/gus* anti-(*Wag*×*A/gus*) F_1 alloantiserum with *Wag* erythrocytes. Complement-mediated cytotoxicity using *Wag* lymph node target cells, measured by ^{51}Cr release. The original titre, ○——○; after absorption with erythrocytes once, △——△; twice, □——□; three times, ●——●; four times, ▲——▲; subsequent absorption with spleen cells, ■——■.

In the experiment described below the question asked is not what class of MHC-directed alloantibody is involved but what is its specificity? It tests the proposition that histocompatibility antibody and hence transplantation antigens as detected by 'tissue typing' may have little to do with transplantation. During the past year evidence has been presented that mixed lymphocyte reactions (MLR)[7] and graft-*versus*-host reactions (GvH)[8,9] may occur in the absence of serological differences in the mouse H-2 system.

The model we have used is that of heterotopic (abdominal) auxiliary heart transplants, anastomosing aorta to aorta and pulmonary artery to inferior vena cava. The method was modified from Ono and Lindsey[10]. The endpoint for rejection was cessation of heart beat, which was somewhat sharper than ECG recordings. Two inbred lines of rates were used which differed at their MHC, *A/gus* (Ag-B.1=H-1^{1}) and *Wag* (Ag-B.2=H-1^{w}). In the experiments quoted (*A/gus*× *Wag*) F_1 female hearts were transplanted to *A/gus* male recipients. Alloantisera against *Wag* rats were raised in *A/gus* rats by immunisation with (*A/gus*×*Wag*) F_1 spleen cells; batches of rats were injected weekly eight times with spleen cells amounting to 10^7 leukocytes at each injection. Such sera generally had cytotoxic titres against *Wag* lymphocytes (^{51}Cr release complement-mediated cytolysis) of about 1/1,000. The sera consistently led to prolonged and usually indefinite survival of heart transplants when given intravenously in six doses of 1 ml each over the first 9 d after grafting. Less than this amount gave irregular results. This situation is illustrated as series *b*, Fig. 1, to be compared with series *a* (normal serum treated controls) which rejected their transplants between 8 and 11 d. Some of the transplants in series *b* and *c* have now survived for more than 250 d. These results agree well with those previously described using this combination of strains[11].

Enhancing serum as used in series *b* was then absorbed to remove Ag-B antibodies but possibly not antibodies against (? serologically silent) products of other genes in the MHC, if such exist. Absorption was monitored by loss of Ag-B cytotoxic titre but we assume absorption is not immunoglobulin-class discriminative and that any non-cytotoxic antibody of the same Ag-B specificity is removed at the same time. There is no precedent for the alternative assumption of immunoglobulin class discrimination in absorption. The source of material for such an absorption is not obvious, but in rodents, erythrocytes carry histocompatibility antigens and there are reasons to suppose that they might not carry other gene products of the MHC (they express no MLR or GvH reactivity). Rat red cells were purified from blood using a closely packed cotton wool column more or less as described by Diepenhost *et al.*[12]. *Wag* blood (500 ml plus anticoagulant) was centrifuged and the cells resuspended in 500 ml of equal volumes of the supernatant plus saline; this was passed through a column 4 cm× 60 cm at 2 pounds per square inch. Fractions were pooled up to two tubes before the first showing any white cells. The purified red cells from one column were sufficient to absorb 100 ml of alloantiserum in three stages until negligible cytotoxicity remained which could not be reduced by further absorption. The absorbed sera were centrifuged at 105,000*g* for 1 h to remove debris before use. Cytotoxicity tests on absorbed and unabsorbed sera are shown in Fig. 2. This red-cell-absorbed serum, used in the same doses as in series *b*, enhanced the transplants at least as well as the original serum (Fig. 1, series *c*).

Cells used for absorption are generally presumed to carry away the bound antibody; it is not obvious, therefore, that soluble Ag-B antigen/antibody complexes would be left in the serum. This is, however, an important point in the interpretation of the results and was checked as follows. Complexes were sought directly by purifying rat IgG (by DEAE ion-exchange chromatography), labelling with ^{125}I and adding this as a marker to a sample of the red cell absorbed enhancing alloantiserum. This mixture was examined on a Sephadex G200 column (Fig. 3) and monitored for ^{125}I γ counts and by rabbit immunoglobulin anti-rat IgG in immunodiffusion assays. The ^{125}I counts located rat immunoglobulin on the column whereas immunodiffusion

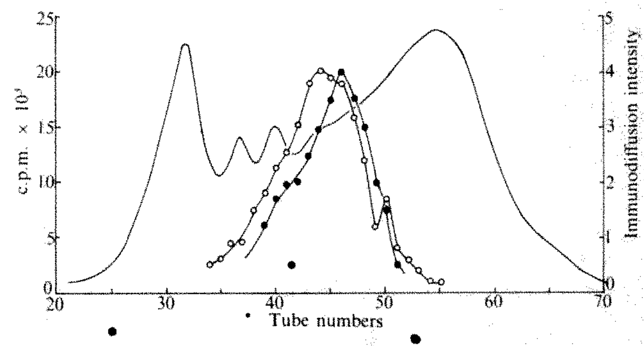

Fig. 3 Sephadex G200 column gel filtration of erythrocyte-absorbed enhancing alloantiserum. Protein profile (280 nm absorption), uninterrupted line. ^{125}I-labelled purified rat IgG added as marker, ○——○. Rat immunoglobulin as tested by a rabbit IgG anti-rat IgG using immunodiffusion, ●——●.

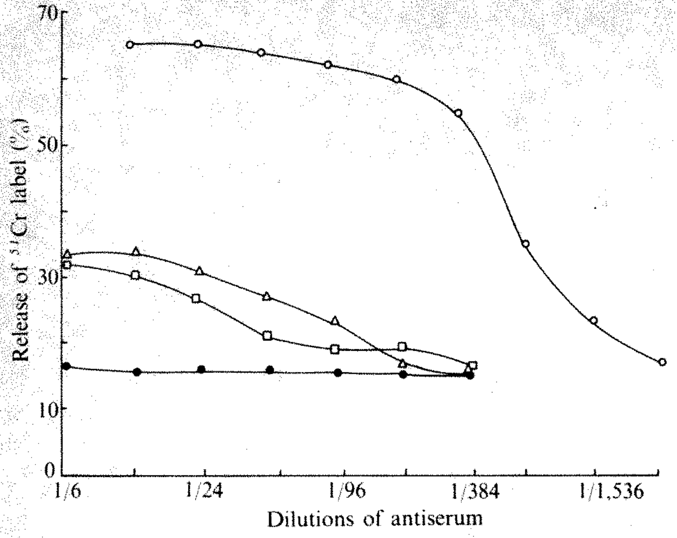

Fig. 4 Complement-mediated cytotoxicity of enhancing alloantiserum. Original titre against *Wag* lymph node target cells, ○——○. After maximal absorption with *Wag* erythrocytes, titre against spleen cells, △——△; against lymph node cells, □——□. There was no measurable reaction (●——●) using as target cell, bone marrow, thymocytes, peripheral leukocytes (unfractionated) or normal peritoneal exudate cells.

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with anti-IgG was expected to locate both free and antigen-bound immunoglobulin. The two peaks showed no differences attributable to antigen–antibody complexes. This method possibly lacks the required sensitivity but shows at least no gross contamination with complexes. Further information was sought by taking red-cell-absorbed enhancing serum (the same batch as used in series *c*, Fig. 1) and reabsorbing with white cells (mixed spleen/thymus used at 5×10^8 cells per ml serum). This treatment would remove antibody of any specificity still present because spleen cells were used for the original immunisation, but their ability to remove complexes by binding the Fc portion of complexed immunoglobulin would presumably be more modest. As can be seen in Fig. 1, series *d*, this doubly absorbed serum had no enhancing activity. These facts support the idea that if complexes are present owing to shedding from erythrocytes they are in small amounts. Because less than 6×1 ml of original enhancing antiserum was inadequate to give consistent permanent heart allograft acceptance, the absorbed serum should not have been effective with its (at least) greatly reduced Ag-B (complexed) antibody content when used at the same dosage, if the transplant protection was Ag-B mediated.

We therefore conclude that Ag-B antibody is not the essential mediator of immunological enhancement, either alone or as a complex, and hence that some antibody of different specificity must be involved. It is possible to imagine that heart allografts in rats are not affected by Ag-B histoincompatibilities or not affected by the Ag-B 1/2 incompatibility in particular. Then rejection could be attributed to multiple non-Ag-B differences. This is unprecedented, which is no good reason for rejecting the idea, but aural/foetal heart allografts in mice obey H-2 rules so we do not give serious consideration to this suggestion. We do not believe that heart-specific alloantigenicity is involved because the enhancing alloantiserum was raised with immunogen which would not carry any such specificity. We suggest that the effective antibody in the absorbed serum is directed against T cell receptor sites and may be an antibody against rat Ir-1$^{\mathrm{w}}$. We favour the view that Ir-1$^{\mathrm{w}}$ gene product from passenger (and resident) leukocytes provides the antigen to complex with the antibody which then blocks the graft recipient's lymphocyte recognition molecules. The implication is that transplant sensitisation is due to donor lymphocytes in the transplanted tissues and any other cells expressing MHC associated gene products. This presumably excludes heart tissue itself.

The residual cytotoxic titre remaining for lymph node cells in red-cell-absorbed enhancing serum (Fig. 2) which cannot be removed by further red cell absorptions and was referred to as negligible is, of course, not negligible. It might be Ag-B antibody for which red cells lack the Ag-B specificity.

The results illustrated in Fig. 4 weigh against such an interpretation; the residual titre can be detected using lymph node cells or spleen cells as labelled target but not using thymocytes, peripheral blood leukocytes, bone marrow cells or normal peritoneal exudate cells. Neither liver nor blood platelets would absorb it out. This is not the cell-type distribution seen for H-2 in the mouse or HL-A in man. Liver is the most effective source of material for absorption of H-2 and HL-A antibodies. This weak cytotoxic effect might not be connected with the enhancing properties of the sera, but if it is, then lack of reactivity by naive thymic lymphocytes is interesting; where might their recognition sites be sequestered until they have escaped thymic influence and been educated?

The serological properties of this absorbed serum are remarkably similar to those described for a proposed Ir-1.1 serum in the mouse[13]. This serum was obtained by cross immunisation of two congenic lines derived from a recombinant such that the only genetic differences lay between *H-2K* and *Ss-Slp*. We propose that absorption with suitable material (for example, red cells, liver, platelets) can also provide a similar class of antiserum, directed at MHC-associated gene products other than those hitherto detected by tissue typing. The role we now ascribe to such antibodies supports the concept of a difference in kind between what have been called SD (serologically defined) and LD (lymphocyte defined)[14] gene products of the MHC.

In summary, we propose that immunological enhancement of transplants is mediated by MHC associated Ir antigen–antibody complexes, which implies that sensitisation to transplants is not due to differences between "transplantation antigens" (as recognised hitherto). On the contrary the differences we invoke are those between recognition molecules of passenger and/or resident lymphocytes in the transplant. Differences between lymphocytes measurable by mixed lymphocyte reactions are responsible for initiating the afferent arc in cell mediated lymphocytotoxicity; transplantation antigens (old style) are involved only in the effector arc[15]. These *in vitro* studies are lymphocyte/lymphocyte interactions and we cannot comment from our present *in vivo* results on whether the rejection process itself, as opposed to sensitisation for rejection, involves the transplantation antigens whose serological markers permit tissue typing.

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Physiology of Grain Filling in Barley

Walpole and Morgan[1] have suggested that differences in absolute growth rates between grains at different spikelet nodes in barley result from differences in quantity of assimilate supplied by each subtending awn, and that this quantity varies in proportion to awn size. They assume implicitly that awns affect grain growth rates by functioning solely as additional assimilate sources. If this is so, then differences in awn size between different nodal positions could determine these growth rate differences only if the assimilate requirements for grain growth were in excess of the total potential pool available for grain growth. Results from other work[2,3] suggest that in barley (as in wheat[4,5]) the converse situation may occur. From studies of temperature effects on the relationship between grain growth and leaf area duration[6,7] and on CO_2 exchange rates[8] in barley, it is a reasonable supposition that under the 'cool greenhouse' conditions used by Walpole and Morgan (with presumably a high light intensity and adequate water supply) the potential assimilate supply may have exceeded the grain growth demand in their plants. Support for this hypothesis is provided by their data as these show no evidence of any competition for assimilate between grains such as might be expected if demand had exceeded supply. If grain growth rates were not limited by assimilate supply, then either awns did not affect them or they did so primarily by affecting potential growth rates independently of assimilate supply, that is, by influencing the 'sink demand' (or 'sink capacity') of each grain.

The determinants of 'sink demand' of individual grains have not yet been satisfactorily resolved. Various suggestions have implicated the number of sites of starch synthesis and storage in each grain (as a function of endosperm cell number[9,10] or cell size[11]) and hormone(s) produced or accumulated within the grains which may directly promote or regulate the accumulation of organic and inorganic substances[9,12–15] and/or influence the duration of synthetic activity in them[12–14]. It is possible too that endosperm size, and therefore the potential number of starch synthesis and storage sites, may be determined partly by the cytokinin content of grains over the period of cell division in the endosperm[15].

Michael *et al.*[12–14] have provided evidence that awns may increase the cytokinin content of grains in barley by increasing the transport of cytokinin from sites of synthesis (probably in the roots) to the grains, as a consequence of increased ear transpiration. Moreover, awn removal reduced grain size at a low air humidity, but not at a high humidity when transpiration was much lower[12–13]. Spraying cytokinin on the ears also increased grain size[13] Differences in grain growth rates and final grain size were associated with differences in cytokinin activity in the grains[14]. This was so whether size differences were genetic in origin or due to treatments such as awn removal or water saturation of the soil. The differences in cytokinin content were apparent before the differences in grain size. There is evidence too that awns may increase the transport of nitrogenous substances into the grains[16].

None of this work provides substantive evidence that awns do affect grain growth rates by affecting 'sink demand', but it indicates in principle that this might occur. For example, during the period of endosperm cell division it is possible that a limiting availability of cytokinin and organic nitrogen compounds (rather than carbohydrates) in the grains frequently may limit volume growth of the endosperm and therefore subsequent growth rate potential during the starch storage phase. Awns of differing lengths, by affecting the quantities of these substances transported into the developing grains, might effectively vary endosperm size so that subsequent growth rates and final starch contents per grain are varied.

It seems likely that assimilate produced in the spike is used preferentially over that from the shoot for grain growth in barley, so that shoot assimilate satisfies demand in excess of spike supply[2,17]. As each spikelet seems to supply assimilate only to its own grain[1,2], and as demand for shoot assimilate seems to be similar at each spikelet node[1], the implication is that if the 'sink demand' of individual grains is increased by increased size of subtending awns, then the additional demand can be satisfied by an increase in assimilate supply from within the spikelet. This might occur in one or more of several possible ways. The increased supply could derive from the longer awn itself. There might also be an increase in photosynthesis rate in the proximal source, that is the whole spikelet, in response to the increased assimilate demand—in the same way that flag leaf photosynthesis rate in wheat has been shown to vary with assimilate demand on the leaf[18]. There is evidence, too, that an enhanced availability of cytokinin in photosynthetic tissue may increase the activity of carboxylating enzymes[19,20]. Photosynthesis rate in the whole spikelet thus might increase with increasing awn size as a result of enhancement by the awns of cytokinin availability in the spikelet tissue[12]. Where the potential supply of assimilate already exceeded grain filling requirements, however, any awn effects on supply from the proximal source would be of secondary importance as any additional assimilate requirement deriving from awn effects on 'sink demand' presumably could be satisfied by increased export from the shoot.

Whether it is more usual for awns of varying size to affect the rate (and/or duration) of grain growth primarily by varying the supply of assimilate to the grains or by varying their 'sink demand' is a question which cannot be resolved until more critical work is carried out. For example, by using DCMU as a photosynthesis inhibitor, awn effects on grain growth could be determined in the presence and absence of awn photosynthesis.

On the basis of currently available information it is probably best to assume provisionally that awns play a multiple role in the determination of grain growth rates and final grain size, with the dominant function depending on genotype and environment. Until more is known of the physiology of the awn-grain relationship it may be premature to specify awn characteristics as selection criteria in breeding programmes for increased yields in barley[1].

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Protein Pellicle of Stigmatic Papillae as a Probable Recognition Site in Incompatibility Reactions

ANGIOSPERM pollen grains carry extracellular proteins in two sites: in the inner wall layer, the cellulosic intine, and in cavities in the outer exine, the sculptured part of the wall composed of sporopollenin[1–3]. The intine proteins, which include several acid hydrolases, are synthesised by the spore and inserted during growth; they are therefore gametophytic in origin. The exine proteins, in contrast, are produced in the tapetum, the nurse tissue of the anther, and injected into the exine cavities during the maturation of the pollen grains; they are thus sporophytic. The enzymic wall proteins seem to be concerned with germination, penetration of the stigma surface and the early growth of the pollen tube. Other fractions are involved in the recognition responses which control breeding behaviour, playing a part in both interspecific[4] and intraspecific[5,6] incompatibility systems.

Protein fractions separated from the exines of species of Cruciferae and Compositae can induce characteristic responses in stigma hair cells. Most notably, extracts from incompatible pollen will elicit a rejection reaction in stigma papillae, a reaction which leads ultimately to the deposition of callose in cells in the immediate vicinity of areas of contact[5]. 'Recognition' in incompatibility systems of this kind presumably depends upon interactions between specific pollen proteins and complementary fractions associated with the stigma or style[7,8]. When the rejection of unacceptable pollen occurs at the stigma surface, as is often found in cases of interspecific incompatibility and in sporophytically controlled intraspecific incompatibility systems, the receptor sites must be in, or upon, the stigma hair cells. In seeking to locate these sites, we have found that the surfaces of the stigma papillae bear an external protein coating overlying the cutinised outer layer of the wall. This coating, which we shall refer to here as the pellicle, has certain properties which lead us to believe that it is functionally important in the capture and hydration of pollen grains. The layer could also be the site of the recognition reactions involved in incompatibility responses.

The principal observations were made on stigmas of *Silene vulgaris* (Caryophyllaceae) and *Brassica oleracea* and *Raphanus sativus* (Cruciferae). In a wider survey, the presence of the pellicle has been confirmed in some eighty other angiosperm families, indicating that it may well be universal in the group. The existence of the layer was first noted when, using fluorescein diacetate as a substrate[9], it was found that esterase activity is associated with a layer lying outside of the cuticularised wall of the stigma papilla. More precise histochemical localisation of non-specific esterase[10] confirmed this distribution. The pellicle was revealed directly with the fluorescent protein stain, 1-anilino-8-naphthylsulphonic acid (1-ANS), used as described elsewhere[5]. Digestion experiments were carried out with Pronase (BDH, 1 mg ml^{-1} in 0.02 M Tris buffer, *p*H 7.2, 1 h at 37° C) and lipase (Sigma Type VII, 2 mg ml^{-1} in 0.1 M Tris buffer, *p*H 7.5, 1 h at 37° C). The benzpyrene technique[11] was used for the localisation of the cuticle underlying the pellicle. For transmission electron microscopy, whole stigmas were prefixed at room temperature for up to 24 h in 1.5% glutaraldehyde in 0.05 M phosphate buffer at *p*H 7.0 containing 12% sucrose, and then transferred to 1% osmic acid in the same buffer for 1–2 h at 3–5° C. The fixed material was embedded in Araldite, and thin sections prepared by standard methods. For scanning electron microscopy fresh or pretreated stigmas were freeze-dried and coated with gold-palladium alloy.

The cleavage of fluorescein diacetate by non-specific esterases releases soluble fluorescein which will normally diffuse from the enzyme site. Some local binding, however, takes place in the pellicle or below it, so the superficial enzyme activity can be detected in a few minutes using this substrate. The esterase activity of the pellicle is, however, best demonstrated using α-naphthylacetate as a substrate in a simultaneous coupling reaction with either Fast Blue B salt[10] or hexazotised pararosanilin[12], where the reaction products are not diffusible. The distribution is the same with the two coupling agents, activity being present over the whole outer surface of the papillae, grading away towards the base (Fig. 1*c*). This distribution coincides precisely with that of a protein layer observable by 1-ANS staining (Fig. 1*a*). So far, no other hydrolytic enzymes have been detected in the pellicle, and it is noteworthy that there is no acid phosphatase activity, although this is always associated with the pollen intine[2].

The protein pellicle ensheaths the cuticle, but without any close attachment. It readily forms cracks and fissures on the older stigma (Fig. 1*c* and *d*), and then the cuticle can be viewed through it after benzpyrene staining (Fig. 1*b*). As comparison of the two scanning electron micrographs (Fig. 1*d* and *e*) shows, the layer is dispersed by Pronase digestion. After its removal the underlying cuticle remains intact, giving the usual cytochemical reactions. So far as can be detected by optical microscopy, lipase treatment does not modify the pellicle.

The stratification of the wall at the tip of a stigma papilla and the location of the pellicle are seen in the electron micrograph of Fig. 1*f*. The plasmalemma is often irregular in this part of the cell. It may adjoin the pecto-cellulosic wall directly, but there are often intervening inclusions with an electron density after standard stain suggesting a proteinaceous nature. The pecto-cellulosic layer is bounded by a cuticle, but this does not form a continuous layer. There are no regular channels of the nature of ectodesmata, but the cutin itself is disposed in bars and rods, with many discontinuities offering communication from the outer face to the underlying pecto-cellulosic wall.

A protein pellicle of the kind described here seems not previously to have been reported from the external surfaces of higher plant cells. Cutinised epidermes frequently bear waxy overlays which enhance their water repellency, but not protein layers which from their hydrophilic nature might be expected to increase wettability. Clearly, however, the presence of a hydrophilic pellicle does help to explain one aspect of the function of stigmatic papillae. The stigmas in many flowering plant families are superficially dry, like those of Caryophyllaceae and Cruciferae, but of course they provide efficient attachment surfaces for pollen; and it is well known that after capture pollen grains become hydrated very rapidly (in *Silene*, for example, volume increases by some 50% in the first 10 min after contact). Three forces are likely to be involved in initiating and maintaining this water movement: capillarity at the surface of contact of pollen wall and pellicle; the hydration of colloids, particularly at the germination apertures of the pollen grain; and, ultimately, osmotic forces as the protoplast of the vegetative cell of the pollen grain begins to vacuolate. The structure of the wall of the stigma papilla suggests that water could be withdrawn through the cuticle underlying the pellicle by way of the discontinuities in this layer, the flow being from the pecto-cellulosic inner wall, which in turn would withdraw water from the turgid protoplast of the stigma hair cell. It may be noted that the mechanism envisaged would have a self-regulatory element. While the hair cells remained turgid, the wall would be distended, the interruptions in the cutinised layer would be gaping, and so the protein pellicle would remain hydrated and presumably adhesive. With incipient drying, such as might be expected in an open flower in the Sun, the stigma hair cells would lose turgidity and shrink; and as the cuticle became less stretched the discontinuities would close, so retarding further water loss. The pellicle would then dry out, and presumably become less receptive. The process would be reversed once the water balance was restored.

Beyond the function of the pellicle in the capture and hydration of pollen, there is the possibility that it might be concerned in pollen-stigma interactions in another way, as the site of the recognition responses of incompatibility systems. It is known that the hydration of the pollen grains upon the stigma is accompanied by the emission of proteins and other mobile fractions carried in the exine and intine[13,14]. The emitted enzymes are probably concerned with germination and the penetration of the stigma surface by the pollen tube[15], and among the non-enzymic protein fractions are those involved in incompatibility reactions[5,6]. Observations on *Silene* and various Cruciferae show that emitted pollen proteins bind to the pellicles of the stigma papillae very quickly after the initial contact, a strong indication that this is the primary recognition site. Significantly, the erosion of the cuticle of the papilla in anticipation of the entry of the pollen tube begins under the binding zones. This might suggest a cutinase 'activation' in the manner proposed by

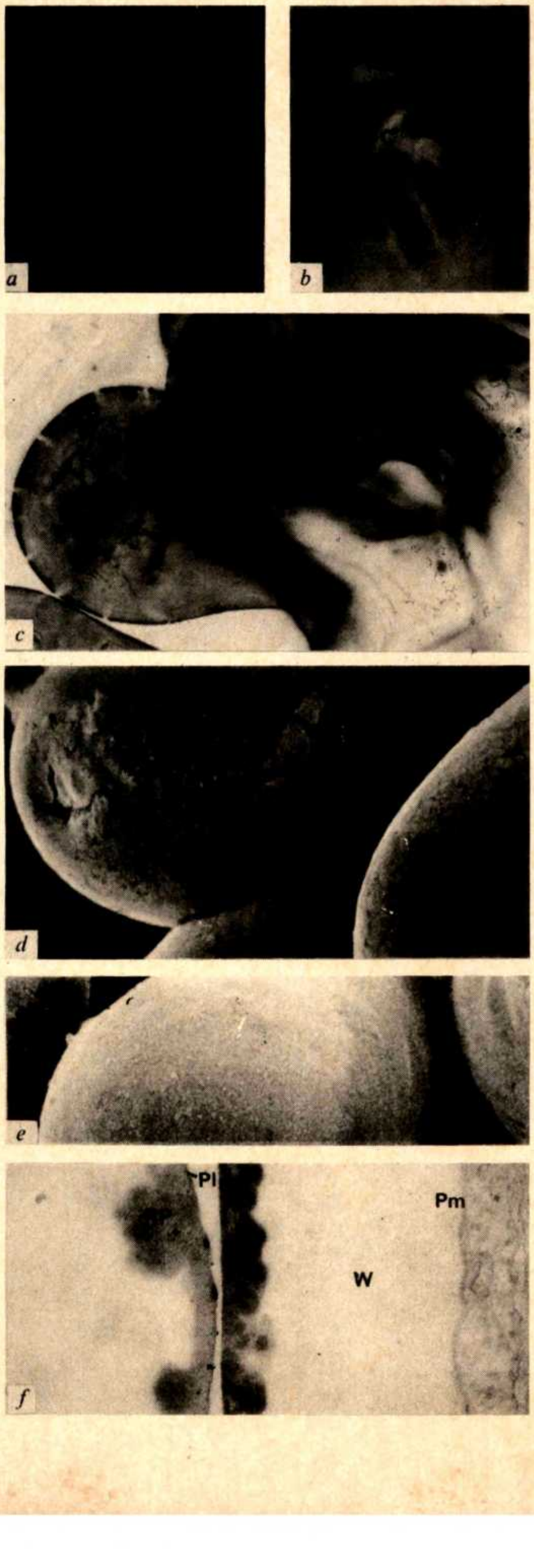

Fig. 1 *a*, Unfixed stigma papillae of *Raphanus sativus* stained *in vivo* with 1-ANS; fluorescence micrograph. The protein pellicle is seen towards the tips of the stigma papillae, about ×750; *b*, stigma papilla of *Silene vulgaris*, esterase reaction with α-naphthylacetate as substrate in a coupling reaction with α-naphthylacetate as substrate in a coupling reaction with Fast Blue B salt, stained also by the benzpyrene method for cutin. The pellicle is seen as a dark sheath investing the cuticle, but where it is torn away the underlying cutin shows the characteristic benzpyrene fluorescence, about ×1,000; *c*, whole stigma papillae of *Silene vulgaris*, esterase reaction as in *b*. The pellicle shows intense activity especially towards the bulbous tips. The discontinuities seen in the profile here are due to cracks and tears, about ×1,850; *d*, stigma papillae of *Brassica oleracea*. Scanning electron micrograph of freeze-dried, unfixed material. The pellicle is clearly seen, with numerous cracks and fissures, about ×3,250; *e*, as *d*, but stigma surface exposed to Pronase digestion. The pellicle has been wholly removed, revealing the underlying cuticle, about ×3,250; *f*, transmission electron micrograph of the wall of a stigma papilla of *Silene vulgaris* towards the tip. The fresh stigma was exposed to an esterase reaction with α-naphthylacetate as substrate in a coupling reaction with hexazotised pararosanilin. The stigma was then fixed in glutaraldehyde and osmicated as in the text. Thin section without post-staining. The pecto-cellulosic wall (W) adjoins the plasmalemma of the papilla (Pm) on the right, with the outer cuticle towards the left (C). The pellicle overlying the cuticle (Pl) is made conspicuous by the associated esterase reaction product (RP) rendered moderately electron opaque by osmication, about ×23,000.

Linskens and Heinen[16], and perhaps offer some clue as to the meaning of the cytochemically detectable 'esterase' activity of the pellicle itself.

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Mitosis in the Cryptophyceae

In a recent issue of *Nature*[1] Oakley and Dodge are critical of my theory on the evolution of the algae[2]. I believe that their criticisms are the result of some misconceptions and inaccuracies. In reference to my article[2] they state "the Cryptophyceae were thought to be ancestral and closely related to the Cyanophyceae". First, there must be some error in sentence structure, for to conclude that the eukaryotic Cryptophyceae are ancestors to the prokaryotic Cyanophyceae is a difficult assumption to make. Second, I never said that the Cyanophyceae were closely related to the Crytophyceae but that the Cyanophyceae were closely related to the chloroplasts of the Cryptophyceae. Therefore one would not expect cell division in the Cyanophyceae to be similar to that in the Cryptophyceae as they infer.

Oakley and Dodge refer to McDonald's work on mitosis in the Rhodophyceae[3] and state that "It (mitosis in the Cryptophyceae) is quite different from that in the Rhodophyceae" without expanding further. I believe that they have overstated their case, as the only differences in mitosis between the two classes are: (1) the presence of some heterochromatin referred to as a kinetochore in the Rhodophyceae with no such structure in the Cryptophyceae, and (2) the presence of basal bodies in the Cryptophyceae and their absence in the Rhodophyceae (which would be expected since the Rhodophyceae have no flagellated cells) although the polar ring[3] in the latter may prove to be a derivative of a basal body. These differences between mitosis in the two classes are not as significant as Oakley and Dodge seem to believe and do not rule out an evolutionary link between the Cryptophyceae and Rhodophyceae.

Lastly, Oakley and Dodge infer that *Chroomonas* (a Cryptophyte with a chloroplast) is a primitive genus in my scheme on the phylogeny of the algae[2]. This is not so as this type of organism is fairly advanced along the evolutionary pathway. If they were seeking a more primitive organism to investigate they should have chosen a Cryptophyte without chloroplasts or one with cyanelles (endosymbiotic Cyanophyceae).

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Drs Oakley and Dodge reply: In regard to Dr R. E. Lee's correspondence, our letter to *Nature*[1] was not intended to be particularly critical of Dr Lee's previous article on endosymbiosis and the evolution of the algae[2]. In fact Dr Lee's theory was only mentioned very briefly. Nevertheless we should like to respond to his comments. Of course when we stated that "the Cryptophyceae were thought to be ancestral and closely related to the Cyanophyceae", there was no intentional implication that the Cryptophyceae were ancestral to the Cyanophyceae but rather that they were ancestral algae. Since Dr Lee's scheme regards them as ancestral to every group of algae other than the Cyanophyceae we feel that it is fair to regard them as ancestral. Although Dr Lee does not state explicitly that the Cryptophyceae are closely related to the Cyanophyceae, his chart (Fig. 1) giving phylogenetic relationships places two groups of cryptophytes, the colourless cryptophytes and those with cyanelles, closer to the Cyanophyceae than any other groups of algae. We do not feel, therefore, that our statement was unfair.

This point, however, is of little importance. A much more important point, and the point we were making, is that Dr Lee's scheme implies that the Pyrrophyta with very little histone[3], extremely unusual base pair composition[4], and an apparently primitive mitotic apparatus[5] has evolved from the Cryptophyceae which show no such apparent primitiveness or uniqueness. Similarly, the mitotic apparatus of the Eugenophyceae seems to be much more primitive than the cryptophytes from which they have evolved according to Dr Lee's scheme.

In fairness, Dr Lee does not feel that the cryptophytes with chloroplasts are ancestral to the Pyrrophyta. He feels that the colourless cryptophytes and those with cyanelles are more primitive than those with chloroplasts. With regard to the colourless cryptophytes, our results were so similar to the light

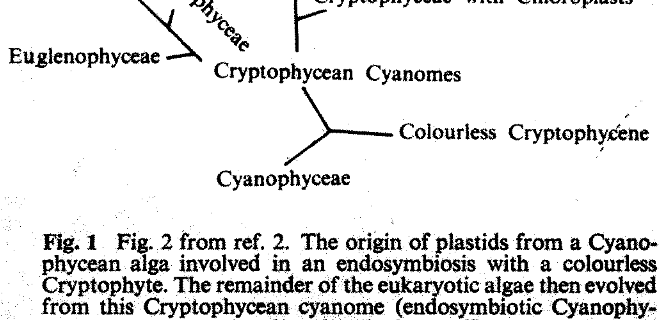

Fig. 1 Fig. 2 from ref. 2. The origin of plastids from a Cyanophycean alga involved in an endosymbiosis with a colourless Cryptophyte. The remainder of the eukaryotic algae then evolved from this Cryptophycean cyanome (endosymbiotic Cyanophycean alga plus the host cell).

microscopic studies on *Chilomonas paramecium*[6,7] that there was no reason to believe that there might be any difference between the coloured and colourless cryptophytes. In addition, there is recent evidence that the colourless cryptophytes possess degenerate chloroplasts[8] and may not be ancestral to the cryptophytes with chloroplasts at all; the reverse being the case.

In regard to *Cyanophora paradoxa* which Dr Lee regards as a cryptophyte with a cyanelle, though this organism certainly seems to possess a cyanelle, there is some question as to its being a cryptophyte. Moreover, mitosis has been studied in this organism[9] and it shows no primitive characteristics.

Finally, Dr Lee does not feel that the presence of kinetochores and the lack of basal bodies in the Rhodophycean alga previously studied[10] are particularly significant, but to us these seem to be quite significant differences. The presence of the unique polar ring and the near complete retention of the nuclear envelope during mitosis in the Rhodophyceae also constitute striking differences. These differences suggest that the mitotic apparatus of the Cryptophyceae is more nearly like that of several other groups of algae than that of the Rhodophyta.

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Secretory discharge and microflora of milk gland in tsetse flies

THE uterine or milk glands in tsetse flies (*Glossina* spp.) are modified female accessory reproductive glands which elaborate and release a nutritive liquid of proteinaceous and lipoid nature for the maturing intrauterine larva[1-3]. The multi-branched tubules of the gland converge into a pair of efferent ducts which fuse inside the oviductal shelf and open into the lumen of the uterus just posterior to the opening of the oviduct[4,5]. Cytological details of the milk gland and their modulations in relation to the state of pregnancy of the female have been described[6] (W-C. M., D. L. D., D. S. Smith and U. Jarlfors, in preparation). Earlier work has suggested that milk is released directly into the lumen of the gland by apocrine secretion[4]. Our observations on the structure of the milk gland do not support such a mechanism, but rather, a novel type of exocrine discharge in which secretion is stored in an extracellular reservoir and released into the lumen through a dense cuticular network. At the points of milk release the lumen is frequently inhabited by bacteria which have not previously been described in milk glands. Our examination is based on milk glands from *G. morsitans morsitans* Westwood, but a comparative study using *G. austeni* Newstead and *G. longipalpis pallidipes* Austen has shown no essential differences among the three species.

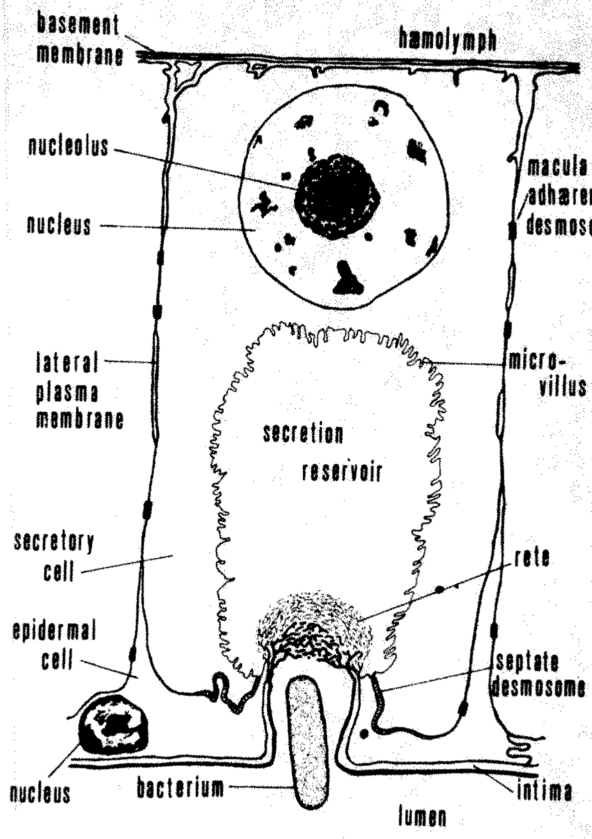

FIG. 1 Schematic representation of the structural organisation of the milk gland tubules in *Glossina morsitans*.

Ultrastructural details were determined by fixation of the gland-fat body complex for 6 h in cold 5% glutaraldehyde buffered at pH 7.4 with 0.05 M sodium cacodylate buffer and post-fixation in buffered 1% OsO_4 for 1½ h. Sections from Epon-Araldite embedded materials were cut on a LKB Ultrotome III using glass knives, mounted on unsupported copper grids, contrasted with saturated uranyl acetate in 50% ethanol followed by lead citrate, and examined with a Zeiss EM 9A electron microscope.

The walls of the gland tubules consist of two layers of cells: a single layer of squamous epidermal cells of ectodermal origin which lines the lumen, and a layer of secretory cells resting on a thin basement lamina. The epidermal layer is continuous with the non-glandular epithelium of the two efferent ducts and has a smooth unlaminated cuticular lining. The efferent ducts themselves have a helically thickened cuticle.

The accessory substances produced by the secretory cells are released by merocrine secretion into an extracellular storage space formed by invagination of the plasma membrane along the apical surface of the gland cell. One such storage reservoir is associated with each gland cell (Figs 1, 2). The stored secretion is channelled into the lumen through a cup-shaped invagination of the intima which projects into the reservoir. At the point of contact with the reservoir the intima is composed of 50–70 nm thick anastomosing strands forming a rete of 2.0–2.5 μm diameter. Underneath this coarse extracellular porous structure the cuticle is further ramified into a mass of extremely fine threads of about 3–6 nm diameter which has a wool-like appearance in the electron micrographs (Fig. 3). This layer is about 1.1 μm thick in the centre. There is no evidence that structural changes or deformations of the ductule occur during cycles of secretory activity. The cuticular wool however has a slightly blurred appearance in the electron micrographs when it becomes saturated with the

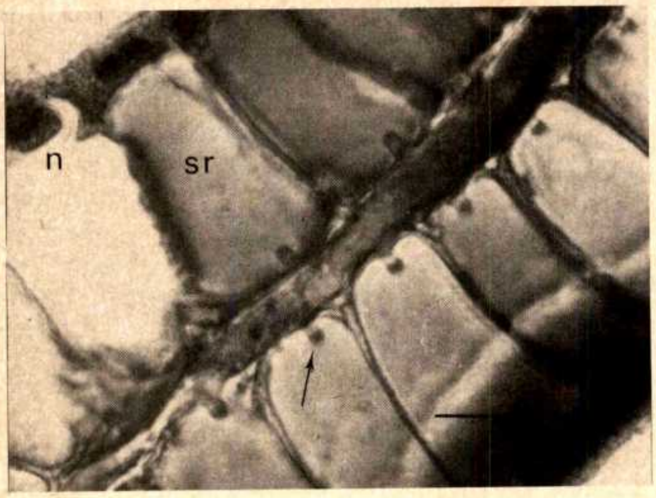

FIG. 2 Longitudinal section through a gland tubule at 6 d after ovulation showing the fully loaded secretory reservoirs (sr), a nucleus (n) of a gland cell, and the ductules projecting from the intima into the reservoirs (arrow). Paraplast embedding, Ehrlich's haemotoxylin-eosin, phase contrast. (Calibration 0.1 mm).

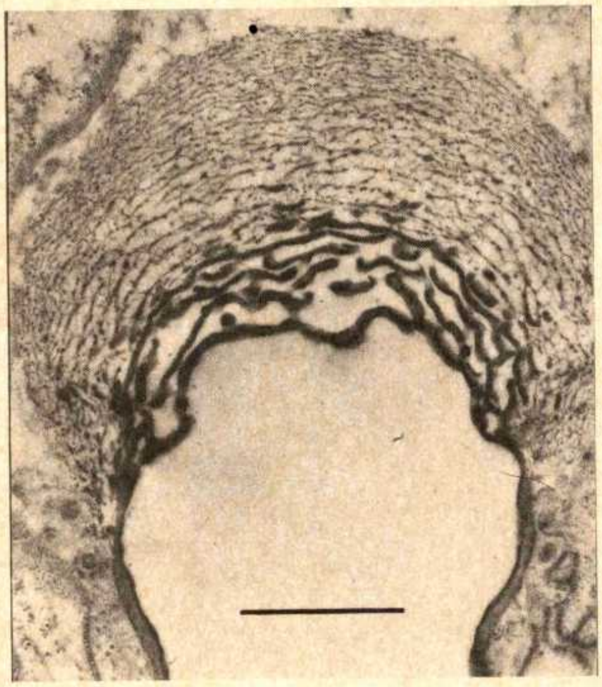

FIG. 3 Electron micrograph of a pore opening in the cuticular intima showing the rete with wool-like cuticular mass. The picture was taken from a secretory inactive gland. (Calibration 0.1 μm).

flocculent or finely granular textured proteinaceous milk secretion during secretory active phases of the gland. The remarkable construction of the pore opening could be functional in providing a high tensile strength, likely to be required to resist stress across the pore opening during cyclic increase in hydrostatic pressure in the reservoirs. Protection against possible rupture of the pore openings during periods of maximal reservoir expansion is further obtained by tight linkage of the plasma membranes of the epidermal cells and the apical surface of the secretory cells by septate desmosomes in the region of the rete. Ruptures have not been observed by us, but an accidental occurrence may account for the apocrine secretion described by Hoffmann[4].

Myofibrils are lacking around the gland tubules, but the 300–350 μm long paired efferent ducts are surrounded by several layers of muscle cells which extend over a length of 200 μm. As illustrated in Fig. 4 the thickness of the muscle layer is at a maximum distally. From a maximum thickness of 20 μm the muscularis decreases in size and disappears completely before reaching the flattened region of the ducts near the oviductal shelf. Circumferential contraction of the muscles therefore produces the greatest force per unit surface area in the distal portion of ducts. Since the glandular part is a closed system the milk can only flow towards the uterus. The high resistance in the narrow and flattened duct region increases the velocity of flow during passage causing the milk to be ejected into the common milk gland duct in the oviductal shelf. At the same time the flattened duct region, also observed in *G. austeni*[7] could function as a valve promoting unidirectional flow in the efferent ducts during alternating muscular con-

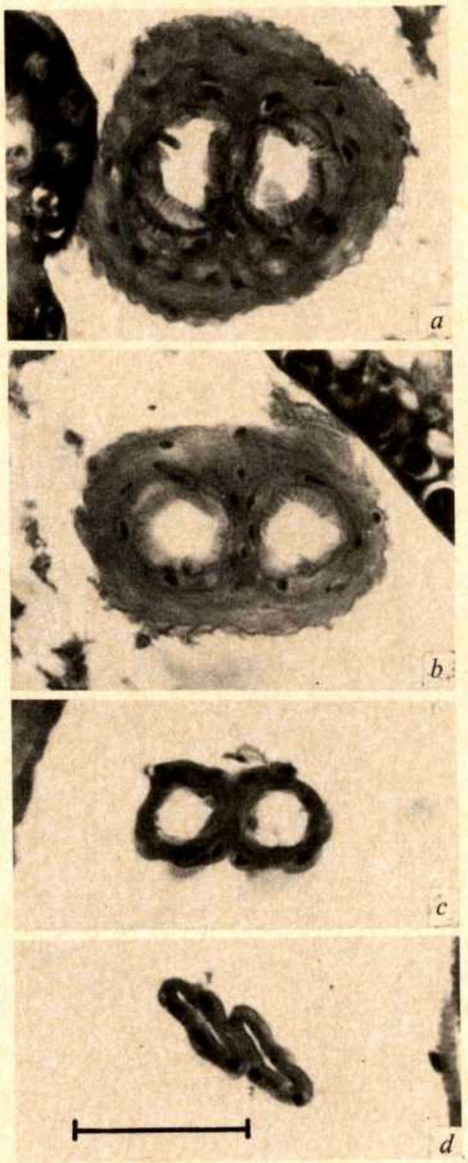

FIG. 4 Cross sections of the efferent ducts taken at intervals of about 30 μm, arranged in order from close to the site of bifurcation into the glandular part *a*, to near the oviductal shelf, *d*. Paraplast embedding Ehrlich's haemotoxylin-eosin. (Calibration 50 μm).

traction and relaxation. Relaxation of the efferent duct muscles after milk ejection would result in a lowering of hydrostatic pressure within the lumen of the gland tubules. The difference in pressure could regulate the outflow of the secretion from the secretory reservoirs into the lumen. The increase of positive hydrostatic pressure within the secretory reservoirs during accumulation of secretion would facilitate outflow, but in itself cannot be responsible for flow regulation. The ejected milk accumulates in the space around the anterior region of the larva, a region which is sealed off from the rest of the uterus by uterine ridges[5]. Suctional forces exerted by the feeding larva could contribute to the amount of milk flow during ejection and may be a prerequisite for its repeated occurrence.

The cuticular rete associated with the pore openings provides an effective barrier against possible invasion of the reservoirs or cells by microorganisms. In fact, the cup-shaped invaginations of the intima are commonly occupied by a rod-shaped, fimbriated type of bacteria (Fig. 5). Whereas these bacteria are numerous in the lumen of the milk gland tubules, they rarely are found in the lumen of the efferent ducts. The bacteria vary in length from 3.4 to 4.5 μm and are 1.1 μm in width. The triple-layered cell wall (total thickness of about 13 nm) consists of two outer dense layers separated by a much less dense layer. A clear intermediate zone of 12–15 nm separates the cell wall from the poorly contrasted plasma membrane (Fig. 6). The cell wall structure coincides with that of Gram-negative bacteria[8], although in the latter the general thickness is about 8 nm. The fimbriae, scattered over the whole cell surface, are 5–7 nm in diameter and about 2 μm long. The bacterial cytoplasm contains scattered electron-dense agglomerations, diffuse filamentous structures, and concentrations of ribosome-like granules. Reproduction is by binary fission following an elongation to about 6.5 μm; a cross-wall is not formed. These properties together with the possession of fimbriae and the absence of mesosomes are commonly shared by Gram-negative, but generally absent in Gram-positive bacteria[9].

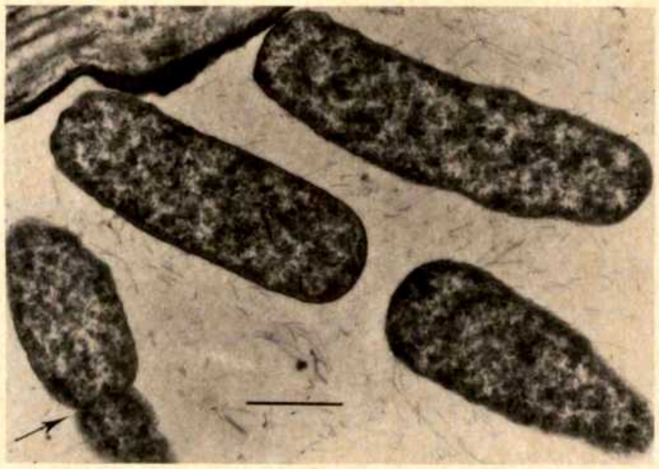

FIG. 5 Electron micrograph of fimbriated bacteria in the lumen of the milk gland. The arrow points to one bacterium during fission. (Calibration 0.1 μm).

The bacteria were observed in the milk gland lumen of all flies ($N = 64$) examined over a period of 1 yr. Since the rabbit population on which the flies were maintained was replaced frequently it seems unlikely that the otherwise perfectly healthy flies could have been infected with a bacteraemia from the rabbits. Moreover, in case of such an infection it would be difficult to imagine how the bacteria could penetrate the gut wall and gland epithelium to reach the milk gland lumen which is lined with cuticle. The bacteria were never observed inside the gland epithelium, nor in the haemolymph or fat body surrounding the gland tubules. Therefore, we interpret the bacteria in the milk gland to be symbiotic microorganisms. It is of interest to note that the symbiotic intracellular microorganisms (bacteroids) occurring in the giant-cell zone of the anterior midgut of *Glossina* have been suggested to be hereditarily transmitted by the maternal secretion from the milk gland[10,11]. The ultrastructure of the gut bacteroids[12] deviates from the gland bacteria in showing a thinner cell wall (though of similar structure), and the absence of fimbriae. But on the basis of these morphological differences alone it is not possible to reject the hypothesis that the fimbriated bacteria represent the free-living form of the intestinal bacteroids. In Salmonellas, fimbriae have been suggested as functional structures for cellular invasion.[13]

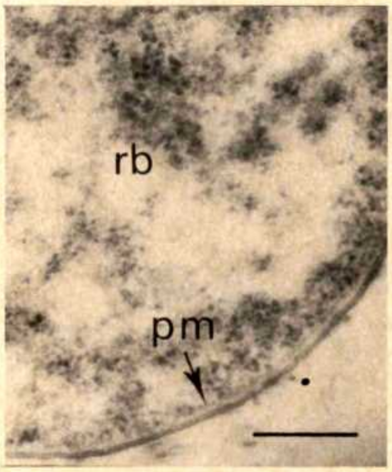

FIG. 6 Part of a symbiotic bacterium with plasma membrane (pm), cell wall, and ribosome-like grana (rb). (Calibration 0.3 μm).

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Vaccination of Rhesus monkeys against Malaria by use of sucrose density gradient fractions of *Plasmodium knowlesi* antigens

We wish to report the results of immunity induction trials in the *Plasmodium knowlesi*-Rhesus monkey system. Thirteen juvenile (1.5-2.0 kg) Rhesus monkeys (*Macaca mulatta*) were vaccinated with various non-viable fractionation products derived from the plasmodial parasite, *P. knowlesi*. Samples were prepared from parasitised erythrocytes by differential French Pressure Cell disintegration and centrifugation, Bio-Gel column chromatographic separation, and linear sucrose density gradient fractionation. Two injections of the respective antigen preparations, emulsified in Freund's adjuvant, were given at 4-week intervals, followed 4 weeks later by blood-stage challenge with the homologous *P. knowlesi* strain. The resulting data reflect a significant difference in peak parasitaemias of the vaccinated monkeys as compared to control monkeys.

Outlined in Fig. 1 and its legend are the preparatory procedures used in obtaining the various parasite fractions which were subsequently tested for antigenic potential. Experimental treatments and results are described in Table 1 and Fig. 2.

The first injection of the immunisation schedule used Freund's Complete Adjuvant (Difco, Detroit) combined with an equal volume of antigen. The second injection, 1 month later, consisted of an equal volume of antigen emulsified in Freund's Incomplete Adjuvant. Groups 1 and 4 were injected each time with 1.0 ml of antigen preparation (average A_{280} 2.0). Groups 2 and 3 received an average of 2.0 ml of the respective antigen preparation (average A_{280} 1.0) at each injection. Concentrations of the respective colloidal antigen preparations used for immunisation were approximated by absorbancy at A_{280}. More exacting measurements were not deemed necessary in view of the relative impurity of the preparations and the linearity of absorbancy to protein concentration. Group 5 received 1.0 ml of Freund's adjuvant at each injection. Injections were given intramuscularly (IM) in opposite thighs.

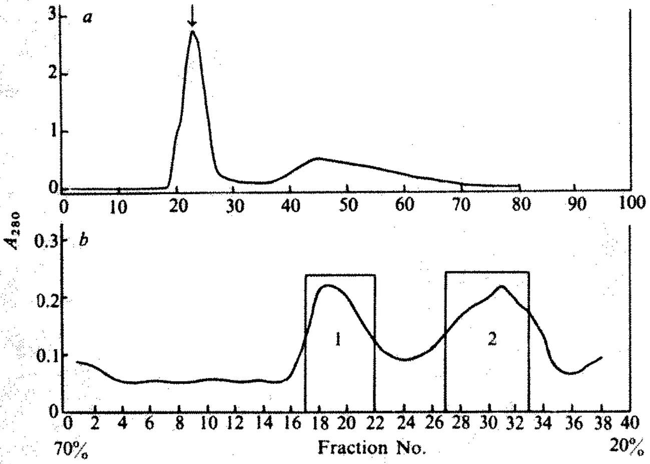

Fig. 1 The first stage of antigen preparation followed the protocol detailed by Schenkel *et al.*[1,5] with the exception that final French Pressure Cell (FPC) effluent was subjected to molecular sieve column chromatography on Bio-Gel A1.5 (Bio-Rad, Richmond, California), rather than on Sephadex G-200. Subsequent fractionation as described in this report focused on the void volume eluate, (arrow, Fig. 1*a*), shown in earlier studies to contain the immunising material, and to be free of detectable host cell contamination[1–5]. Fractionation of the Bio-Gel void volume eluate was accomplished by linear sucrose density gradient centrifugation[6]. The gradients, with terminal concentrations of 20–70%, were prepared by means of a gravity flow apparatus at 4° C. Each cellulose tube contained a total of 29.0 ml. Centrifugation (at 25,000 r.p.m. for 240 min at 4° C) was carried out in the Beckman Model L ultracentrifuge, equipped with a SW 25.1 head (Beckman Spinco Division, Palo Alto, California). Samples (0.7 ml) were collected by means of a fractionator using needle puncture of the gradient tubes. A typical profile is seen in Fig. 1*b*. Vaccination preparations consisted of respectively pooled peaks 1 and 2. Sample pools were dialysed overnight against PBS (2-3 changes) to remove the sucrose.

Table 1 Treatment, survival, and peak parasitemias of vaccinated and control monkey challenged with *Plasmodium knowlesi*.

Treatment	Group No.	Monkey No.	Monkey surviving challenge = +	Peak parasitemia after initial challenge
Bio-Gel void volume eluate preparation in Freund's adjuvant	1	1486	+	<0.5
		1481	+	7.5
		1493	+	2.5
Peak 1 sucrose density gradient fraction in Freund's adjuvant	2	1484	–	0.0
		1489	–	0.5
		1491	+	2.5
Peak 2 sucrose density gradient fraction in Freund's adjuvant	3	1483	–	6.0
		1492	+	<0.5
		1497	+	<0.5
Bio-Gel void volume eluate-no adjuvant*	4	1476	–	50.0
		1477	–	58.0
Freund's adjuvant-no antigen	5	1479	–	24.0
		1480	–	48.0

* In earlier published data[1] the void volume eluate without adjuvant had been shown to be non-protective, thus, this group constituted an additional control.

The challenge inoculum consisted of approximately 2×10^7 *P. knowlesi*-infected red blood cells, given intravenously 1 month after the second antigen injection. The progress of the infection was monitored by percentage parasitaemias, red blood cell counts, and microhaematocrits. (Due to the fulminant nature of the infection, determination of exact parasitemias at death was difficult to obtain. For this reason, the observed parasitemias of unprotected animals may have been, in many cases, artificially low.)

The significance of differences in mortality rates was calculated by Fisher direct *P* significance test.[7]

All surviving monkeys were rechallenged with 1×10^8 parasitised RBC of the homologous *P. knowlesi* strain 3 months after the initial challenge. At that time the red blood cell counts and microhaematocrit values of these animals had returned to prechallenge levels, and no parasites were detectable in peripheral blood smears.

The monitored responses to the initial challenge can be generally categorised into three classes: 6/9 vaccinated animals (Groups 1, 2 and 3) which demonstrated parasitaemias of 0-7.5% and survived; 3/9 vaccinated animals (Groups 1, 2 and 3) which demonstrated similar parasitaemias, but did not survive; and, 4/4 control animals (Groups 4 and 5) which demonstrated extremely high parasitaemias of up to nearly 60% and died.

In Group 1, all three animals survived challenge with the average maximum parasitaemia being 4%. In Group 2, two of three animals died after challenge, although the parasitaemias of all animals in this group were extremely low (average maximum parasitaemia, 1.3%). At necropsy one of the two non-surviving monkeys (1489) showed evidence of a respiratory infection. Two of three monkeys in Group 3 survived challenge. The parasitaemias of all animals were very low and that of the survivors did not exceed 0.5%.

A wide range of indirect evidence[8] suggests that Peak 1 contained primarily the malarial pigment, haemozoin, together with protective antigenic material. Peak 2 seemed to be essentially free of iron, as assessed by atomic absorption spectrophotometry, and contained protective antigenic material. Thus, it had been anticipated that both sucrose gradient peaks would confer protection against a challenge of *P. knowlesi*.

All animals in Groups 4 and 5 died of a rapidly fulminating infection, reflecting the normal course of the disease in nonprotected Rhesus monkeys. The parasitemias and RBC counts of these monkeys were indistinguishable from the numerous non-injected controls used in previous experiments. The lack of protection of animals in Group 4, which received Bio-Gel void volume eluate without adjuvant, corroborated our earlier experiments and those of other investigators[1,9,10], and emphasised the importance of combining the antigen with an effective adjuvant. Group 5 represents a control to detect non-specific resistance induced by Freund's adjuvant alone. No such resistance was observed.

The peak parasitaemias of the nine animals in experimental Groups 1, 2 and 3 were similar, whether or not the animals survived, and were significantly lower than those of the animals in the control Groups 4 and 5 (Table 1). Figure 2 indicates that the course of the anaemia was similar in all animals.

Upon rechallenge none of the surviving monkeys succumbed to the infection and peak parasitaemias were not in excess of 1%.

These results confirm and extend the earlier findings of Schenkel *et al.*[1,5] concerning the feasibility of vaccinating sub-human primates. However, one of the more striking observations concerns the drop in red blood cell profiles following challenge. After a slight initial rise in both RBCs and microhaematocrits, the blood indices dropped dramatically, even in protected groups where parasitaemias remained <5% or were entirely undetected. A similar but smaller decline was observed upon rechallenge of surviving animals.

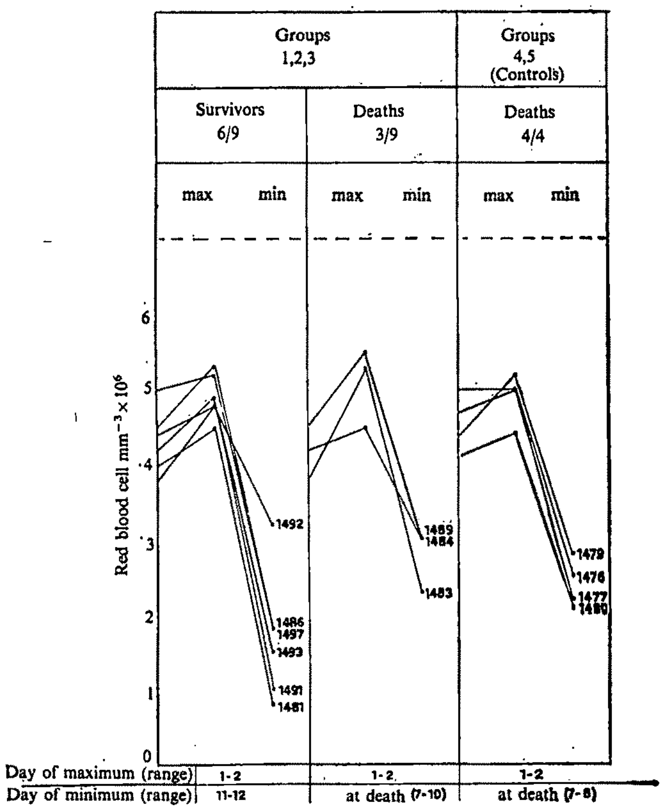

FIG. 2 Maximum and minimum RBC counts following initial challenge. Animals are divided into the three defined classes (vaccinated-surviving; vaccinated-fatality; control).

The aetiology of the anaemia could be postulated as due to some complication of the vaccination process and/or the natural course of the infection, as erythrocyte destruction greater than that predicted from the percentage of cells infected has been well documented[11–18]. Also, certain factors of the present experimental design may be relevant. Unlike previous trials, in this experiment juvenile Rhesus monkeys (1.5 kg weight), rather than adult animals (6 to 7 kg), were used, thus more closely simulating the immune status of children, and the challenge inocula were extremely status of (Analysis of minimum infective dosage in the *P. knowlesi*-Rhesus monkey system has demonstrated that the number of parasitised erythrocytes sufficient to cause death approaches five: L. Schmidt, personal communication.) The contributions of each of these parameters, or combination of parameters, to the observed haemolysis is unkown.

In this study, mortality figures (antigen in Freund's adjuvant versus control groups) become significant to less than 1% only when the results of the preceding vaccination series[1,5] are included. Summation of the data produces a total series of twenty five monkeys. Eleven of seventeen vaccinated animals have survived challenge, while none of the eight control animals have survived ($P < 1\%$).

In summary, we have demonstrated that a significant decrease in parasitaemia and an increase in survival of Rhesus monkeys infected with *P. knowlesi* can be induced by the administration of non-viable sucrose density gradient fractions, in Freund's adjuvant, of the void volume preparation used successfully in earlier studies[1,5].

We thank Drs B. DeLannoy and F. Beluhan, Medical Research Laboratory, University of Illinois Medical Center, Chicago for maintaining the monkeys. This work is supported by the Agency for International Development, United States Department of State.

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Binding of thrombin to human platelets

MUCH work has been carried out aimed at elucidating the mode of action of thrombin on platelets[1–5]. These studies were based on the assumption that hydrolysis of a protein by thrombin constitutes the primary event culminating in aggregation of platelets. Results of recent studies suggest that the primary step in this process might be binding of thrombin to a receptor site, triggering viscous metamorphosis of the platelets[6,7]. The processes of binding and proteolysis need not be mutually exclusive, however, binding may be followed by proteolysis. Here I present direct evidence of thrombin binding by human platelets and discuss some of its implications.

Human blood from volunteer donors was collected in acid-citrate-dextrose in plastic bags and the red cells were removed by differential centrifugation. The platelet-rich plasma (PRP) was recovered in a plastic bag in less than 2 h after collection. Siliconised glass or plastic materials were used throughout this study. The platelets from PRP were isolated and washed by the density gradient method using sodium metrizoate (Nyegaard Co., Oslo) as a cushion[8]. The final volume of the washed platelets in modified Tyrode's solution was adjusted so as to restore the platelet concentration to approximately that of the original PRP.

Bovine thrombin (Parke Davis, Detroit, Michigan) was purified by ion exchange chromatography on SP-Sephadex C-50 (ref. 9). The specific activity varied between 1,200 to 1,700 units mg^{-1} of protein. Experiments using US Standard human thrombin (NIH, lot H-1) gave essentially similar results. The thrombin was labelled with ^{125}I following the ICl method of McFarlane as developed for fibrinogen[10]. The labelled thrombin clotted fibrinogen and aggregated platelets in a way similar to unlabelled thrombin. The label was found to be unstable and the sample was dialysed against 0.1 M Tris-0.1 M NaCl, *p*H 7.2 before use. Because of this elution problem, the label simply serves the purpose of following the protein to which thrombin might bind and is not suitable for quantitative estimations at this stage. The activity of thrombin was measured from clotting time of human fibrinogen (Grade L, Kabi, Sweden) with respect to a standard preparation.

All experiments were done in duplicate and the data presented are average values. To 10–100 µl of labelled thrombin in each plastic conical centrifuge tube, 10 ml of washed platelets in modified Tyrode's solution was added. The tubes were capped, rapidly inverted once and then held at room temperature for 15 min. Depending on the experimental conditions and the amount of thrombin present, the platelets in some of the tubes aggregated. The samples were centrifuged in a swing out rotor (International Centrifuge Co., Model CS, Massachusetts) at 2,500 r.p.m. for 10 min. The platelet sediments were washed once with physiological saline in the same conditions. Preliminary experiments showed that further washing did not make any significant difference in the results. The supernatant, the wash and the platelets were counted in a Packard Auto Gamma Spectrometer. A significant amount of radioactivity was found to be bound to the platelets (Fig. 1). It is interesting to note that a saturation point was not reached within the range studied even though aggregation occurred at a much lower concentration of thrombin. It seems that as the concentration of the added thrombin increases, the number of receptor sites also increases, suggesting transformation of the membrane with unmasking of new binding sites.

Previous studies with thrombin-sensitive proteins were generally done with platelets washed by the method of differential centrifugation. The platelets are fragile and it is now well recognised that this method of handling causes considerable and variable damage to the platelets. This

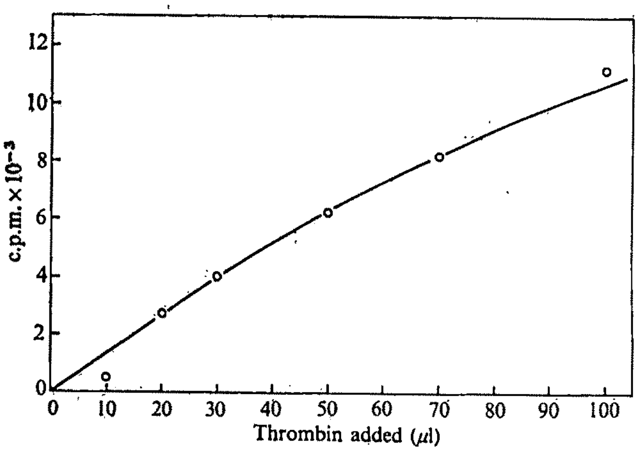

FIG. 1 Binding of thrombin by washed platelets. The platelets were isolated and washed by the metrizoate method and suspended in a modified Tyrode's solution at a concentration of approximately 30,000 μl^{-1}. The labelled thrombin solution had 20 units ml^{-1}.

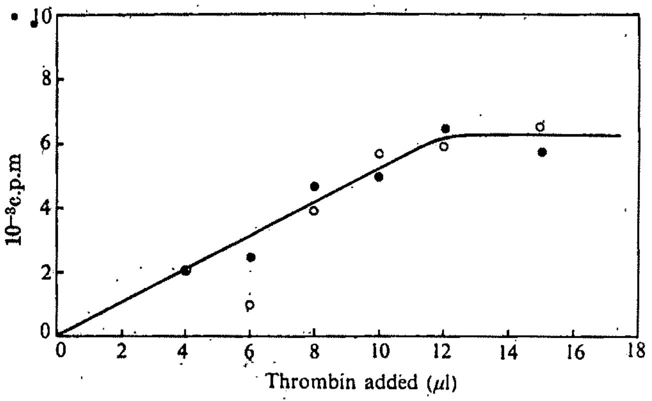

FIG. 2 Binding of thrombin by platelets in plasma. Platelet-rich plasma (10 ml) was incubated with different amounts of labelled thrombin. The platelets were collected by centrifugation, washed and counted. The thrombin solution has 30 units ml^{-1}. ○, Control; ●, aspirin (1mg ml^{-1} final concentration).

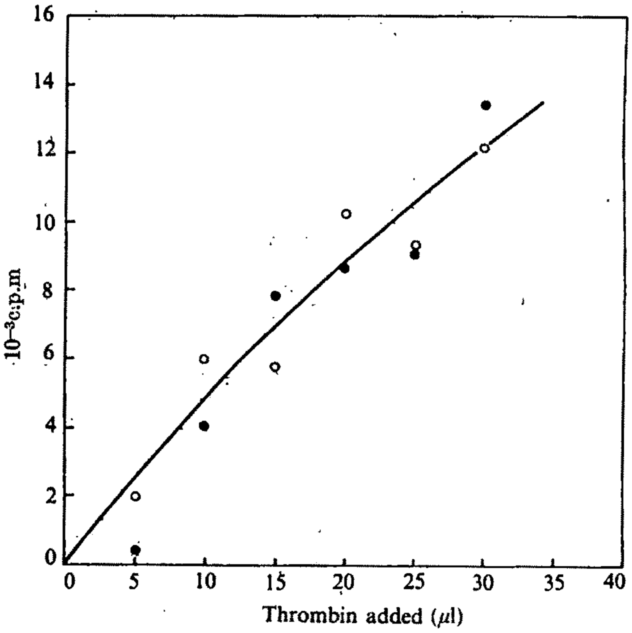

FIG. 3 Effect of PMSF on the binding of thrombin by platelets in plasma. The experimental procedure was similar to that for Fig. 2. The thrombin solution had 3 units ml^{-1}. ○, Control; ●, PMSF (1 mg ml^{-1} final concentration).

might conceivably make normally inaccessible protein components available for nonspecific action of thrombin. Although the metrizoate method is more gentle than differential centrifugation, it was considered that it should be possible to extend these binding studies to platelets in plasma. There is a critical range, generally in the area of 0.2–0.8 units ml^{-1} final concentration, of thrombin which will aggregate the platelets without clotting fibrinogen. For each experiment, this critical value of thrombin was first determined by studying the aggregation behaviour of the platelets in plasma in an aggregometer. The PRP platelets were then treated with thrombin concentrations below this critical value.

The experimental procedure was similar to that described above for washed platelets, except that the time of incubation was 2 to 10 min. Again a substantial amount of thrombin was found to be bound to the platelets (Fig. 2). With platelets in plasma however, a saturation point was attained. Blood coagulation, haemostasis and thrombosis are complicated processes involving the interaction of several factors, a number of which are thrombin-sensitive. The possibility that additional receptor sites became available due to handling and washing of platelets should also be entertained. It was further argued that it should be possible to extend these binding studies to whole blood without any handling and separation. At this point, it is difficult to obtain precise quantitative data from these experiments because of technical problems in achieving clear separation between plasma, platelets and red cells. My results do, however, show that the platelets bind thrombin preferentially. On a per cell basis, the red cells bind little, if any, thrombin.

It is well known that aggregation of platelets with low concentrations of thrombin is inhibited by aspirin. It has also been reported that phenylmethylsulfonyl fluoride (PMSF) inhibits the clotting activity of thrombin although there is very little effect on the esterase activity[9]. Thrombin treated with PMSF fails to induce platelet aggregation. It was considered worthwhile to investigate the effect of these two inhibitors of platelet aggregation on the thrombin binding capacity of the platelets. From Figs 2 and 3, it can be seen that neither aspirin nor PMSF affect the binding of thrombin to platelets. It must therefore be concluded that binding of thrombin in itself is not sufficient to cause aggregation of the platelets and that one or more additional steps are involved. Similar results have been obtained using diisopropyl fluorophosphate (DFP) treated thrombin[11].

The platelet buttons from the above control experiments were suspended in 0.1 M Tris, 0.25 M NaCl, 1% Triton X-100, *p*H 7.5. The suspensions were vortexed repeatedly and then centrifuged at 12,000 *g* for 10 min. Most of the platelet material went into solution, however, a small amount of sediment containing appreciable radioactivity was obtained. Here I deal with the soluble part only. The supernatant was analysed by disc electrophoresis at 5% or 7% gel concentrations. A single peak containing radioactivity was observed at the origin (Fig. 4). The stained gels also showed protein material in this area. Analyses of control samples showed that this peak is not due to thrombin itself. Several supernatant samples from the electrophoresis experiment were pooled and dialysed overnight against Tris-NaCl-Triton. The sample filtered through a column of Sephadex G-200 showed one main peak at void volume followed by a shoulder, containing radioactivity (Fig. 5).

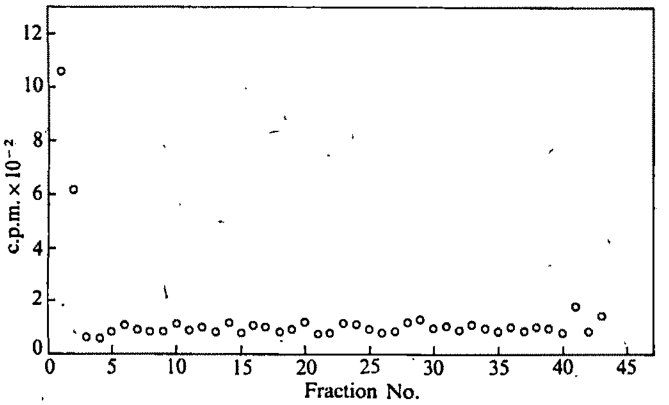

Fig. 4 Distribution of radioactivity after gel electrophoresis. Platelets were treated with labelled thrombin as in Fig. 1. The washed platelet button was dissolved in 0.05 M Tris, 0.25 M NaCl, 1% Triton X-100, *p*H 7.5 and electrophoresed in 5% acrylamide gel. The gel was cut into 2 mm sections and counted.

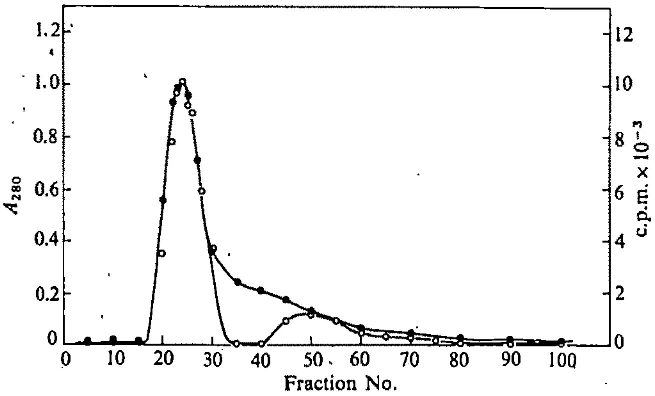

Fig. 5 Variation of absorbance (○) at 280 nm and of radioactivity (●) after gel filtration. Platelets were treated with labelled thrombin, washed and dissolved in Tris—NaCl—Triton X-100. After centrifugation and dialysis, the sample was applied to a column (2.5 cm × 40 cm) of Sephadex G-200 equilibrated with 0.05 M Tris, 0.25 M NaCl, 0.1% Triton X-100, *p*H, 7.5. Fraction size 2 ml.

The following conclusions may be drawn from these results. Human platelets bind thrombin tightly; this binding is specific and preferential compared with other blood components. Binding of thrombin *per se* is not sufficient for platelet aggregation and other steps are involved. With washed platelets, the number of receptor sites is dependent on the concentration of thrombin used indicating transformation of the membrane. In human platelets the receptor of thrombin seems to be a protein with a molecular weight of over 200,000.

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Vitamin B_{12} antagonism by monocarboxylic acids and anilides of cyanocobalamin

VARIOUS test systems have been used to examine the antimetabolite activity of vitamin B_{12} analogues[1]. In general, the tests have relied on the demonstration of growth inhibition or depression in either vitamin B_{12}-requiring microorganisms or vitamin B_{12}-deficient experimental animals. I have investigated the effect of the mixed isomeric monocarboxylic acids of cyanocobalamin and the anilides derived from these acids on the *in vivo* biochemical function of vitamin B_{12} in vitamin B_{12}-depleted baboons.

Seven male baboons *(Papio cynocephalus cynocephalus)* weighing between 10 and 15 kg were used. They had been fed on a vitamin B_{12}-deficient diet consisting of starch, sucrose, vitamin-free casein and maize oil, with salt and vitamin supplements, and although they were haematologically normal, by comparison with baboons fed the same diet supplemented with 2 µg vitamin B_{12} per baboon per day, they had low serum and liver vitamin B_{12} levels and excreted increased quantities of methylmalonic acid (Table 1). The vitamin B_{12}-deficient diet was fed throughout the study, but the daily folic acid intake which had previously been 1 mg was reduced to 0.1 mg. The analogues were administered intramuscularly as sterile solutions in saline. The monocarboxylic acid was given to three baboons at a daily dose rate of 500 µg per kg body weight for 1 month and at a daily dose rate of 250 µg per kg body weight for a further 2 months. The anilide was given to one baboon at a daily dose rate of 250 µg per kg body weight for 3 months. The three remaining baboons served as controls and received daily injections of saline. Vitamin B_{12} and folic acid levels were measured microbiologically with *Lactobacillus leichmannii*[2] and *casei*[3] respectively. Methylmalonic acid (MMA) was measured by thin layer chromatography[4] and formiminoglutamic acid (Figlu) enzymatically[5]. Haematology was carried out on a Coulter counter model ZBI.

Both analogues produced similar effects. The most marked effect was on the excretion of MMA. In vitamin B_{12} deficiency there is an increase in the amount of MMA excreted in the urine due to a reduction in the tissue concentration of 5′-deoxyadenosyl-B_{12} which is a cofactor in the conversion of methylmalonyl CoA to succinyl CoA[6]. Valine, a metabolic precursor of methylmalonyl CoA, causes an increase in the amount of MMA excreted in vitamin B_{12} deficiency[7]. Before treatment started, all seven baboons excreted increased amounts of MMA after a loading dose of 5 g L-valine given intraperitoneally. Treatment with either the monocarboxylic acid or the anilide caused an approximately ten-fold increase in the amount of MMA excreted (Fig. 1) and it remained higher than that in the control animals for some months after treatment stopped.

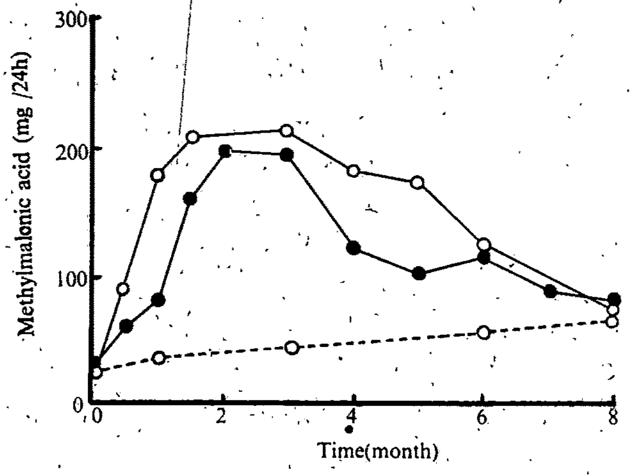

FIG. 1 Methylmalonic acid excretion after 5 g L-valine (intraperitoneal) by vitamin B_{12}-depleted baboons which were given intramuscularly for 3 months either the monocarboxylic acids (○–○, mean of three animals) or anilides (●–●) of cyanocobalamin; and in control baboons given saline (○– – –○, mean of three animals).

Apart from the isomerisation of methylmalonyl CoA, the only other reaction in mammalian tissues known to require vitamin B_{12} is the transfer of a methyl group from N^5-methyltetrahydrofolate to homocysteine to form methionine and tetrahydrofolate[8]. In vitamin B_{12} deficiency serum folate levels tend to be high[9] and there is an increase in the excretion of Figlu[10]. It has been proposed that the former is due to a build up of N^5-methyltetrahydrofolate and the latter to the consequent reduction in the concentration of tetrahydrofolate which is required for the conversion of Figlu to glutamic acid. The amount of Figlu excreted after an oral dose of 5 g L-histidine increased in all three baboons treated with the monocarboxylic acid and also in the baboon treated with the anilide (Fig. 2). The increase, however, was only small and returned to normal soon after treatment stopped. The serum folate levels in the three baboons treated with the monocarboxylic acid were higher (3.2, 4.2 and 3.2 ng ml^{-1}) than in the three control animals (2.3, 2.4 and 2.2 ng ml^{-1}), but not in the baboon treated with the anilide (2.0 ng ml^{-1}).

TABLE 1 Comparative data for baboons fed vitamin B_{12}-deficient diet or vitamin B_{12}-supplemented diet

	Vitamin B_{12}-deficient (7)*	Vitamin B_{12}-supplemented† (3)*
Serum vitamin B_{12} (pg ml^{-1})	19 ± 3.7	219 ± 39.8
Serum folate (ng ml^{-1})	5.3 ± 0.59	5.9 ± 0.78
Liver vitamin B_{12} (µg g^{-1})	0.32 ± 0.03	1.07 ± 0.20
Methylmalonic acid excretion‡ (mg per 24 h)	24 ± 1.6	1 ± 0.4
Formiminoglutamic acid excretion§ (mg per 8 h)	20 ± 4.1	16 ± 1.5

The results are means ± standard errors.
* No. of animals
† 2 µg d^{-1}
‡ After 5 g L-valine given intraperitoneally
§ After 5 g L-histidine given orally

The megaloblastic anaemia seen in vitamin B_{12} deficiency in man has not so far been reproduced in vitamin B_{12}-deficient experimental animals. The haematological parameters of the baboons treated with either the monocarboxylic acid or the anilide remained unchanged during the first 2 months. At this time anaemia was induced by removing approximately 400 ml blood over a period of 7 d. Iron was administered intramuscularly to prevent iron deficiency. The rate of red blood cell regeneration was lower in the treated animals than in the controls (Fig. 3). There was, however, no increase in the mean cell volume suggesting that the mechanism by which the analogues depress the rate of red blood cell regeneration is not the same as that producing the anaemia of vitamin B_{12} deficiency in man. Csanyi *et al.*[11] found that the administration of cyanocobalamin monocarboxylic acid produced leucopenia in rats. This was not so in my study; both the number of white blood cells and platelets remained normal.

The increase in the excretion of MMA suggests that both the monocarboxylic acid and the anilide exerted an antagonistic effect on the function of vitamin B_{12}. The results however shed no light on the mechanism of their action. One possibility is that they might compete with and displace vitamin B_{12} from the tissues. This would result in an

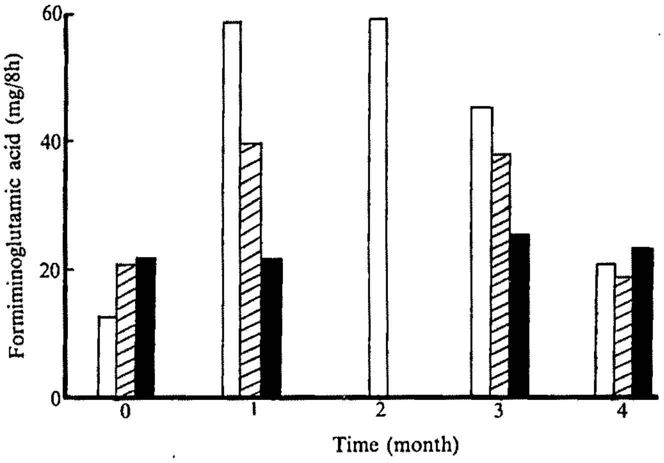

FIG. 2 Formiminoglutamic acid excretion after 5 g oral histidine by vitamin B_{12}-depleted baboons given intramuscularly for 3 months either the monocarboxylic acids (▨, mean of three animals) or anilides (☐) of cyanocobalamin and in control baboons given saline (■, mean of three animals).

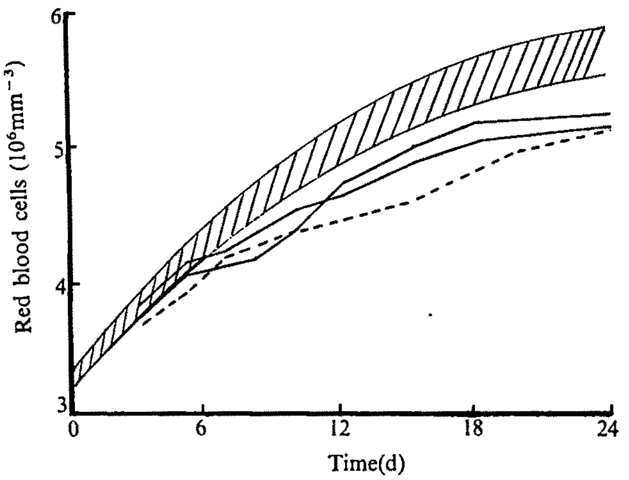

FIG. 3 Red blood cell regeneration in vitamin B_{12}-depleted baboons given intramuscularly either the monocarboxylic acids (——) or anilides (- - -) of cyanocobalamin. The shaded area represents the red blood cell regeneration in control baboons treated with saline.

increase in the serum vitamin B_{12} level. Unfortunately, both analogues supported the growth of *L. leichmannii*, the microorganism used for estimating vitamin B_{12}; 1 μg monocarboxylic acid caused growth equivalent to 0.16 μg vitamin B_{12} and 1 μg anilide caused growth equivalent to 0.029 μg vitamin B_{12}. It was not surprising, therefore, to find a large increase in the apparent serum vitamin B_{12} levels of the treated animals. The levels in the baboons treated with the monocarboxylic acid were greater than 2,000 pg ml^{-1} and in the baboon treated with the anilide it was 323 pg ml^{-1}. Measurement of the concentration of vitamin B_{12} in the liver, 3 months after treatment stopped, was also inconclusive since the levels in the three baboons treated with the monocarboxylic acid (0.18, 0.19 and 0.15 μg g^{-1}) and in the baboon treated with the anilide (0.21 μg g^{-1}) were similar to the levels in the control animals (0.15, 0.16 and 0.23 μg g^{-1}).

It has been suggested that the accumulation of methylmalonic acid may be responsible for the neuropathological changes seen in vitamin B_{12} deficiency in man[12]. Although I did not undertake detailed neurological studies, none of the baboons developed any physical signs of neurological impairment.

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Group analysis of volatile and non-volatile N-nitroso compounds

THERE is much concern about the possible widespread occurrence of N-nitroso compounds in the environment[1]. Many N-nitroso compounds are known to be carcinogenic[2,3], and some 'volatile' nitrosamines have been found in cured food products such as smoked fish[4–6], cooked bacon (ref. 5 and United States Department of Agriculture News Release, 1972), and sausages[6]. N-nitroso compounds are generally unstable and have a wide range of physical properties, particularly with respect to volatility and solubility.

Analytical methods are available for the fourteen most volatile nitrosamines when they are present at the μg kg^{-1} level in the original foodstuff[7]. The procedures involve concentration and clean up by distillation and extraction, followed by separation by gas-liquid chromatography (GLC) and detection by either the Coulson conductivity or the flame ionisation GLC detectors. Because they are non-specific to N-nitroso compounds, however, the GLC detectors are useful for screening purposes only; confirmation by GLC-mass spectrometry is still mandatory[8,9].

Judging from the wide variety of complex secondary amines occurring in nature, a large group of naturally occurring high molecular weight N-nitroso compounds must be expected. As a result of their high molecular weight, they would be 'non volatile', and not amenable to clean up by steam or vacuum distillation. For this reason methods are not yet available for the analysis of non-volatile N-nitroso compounds, even at mg kg^{-1} levels.

Here we describe a group-specific N-nitroso compound analyser which is capable of detection at the μg kg^{-1} level without preconcentration or purification. A detailed description of the experimental apparatus, including flow diagrams and associated electronic circuitry, is presented elsewhere[10]. The N-nitroso compound, dissolved in dichloromethane (DCM), is injected into a flash heater and swept by argon

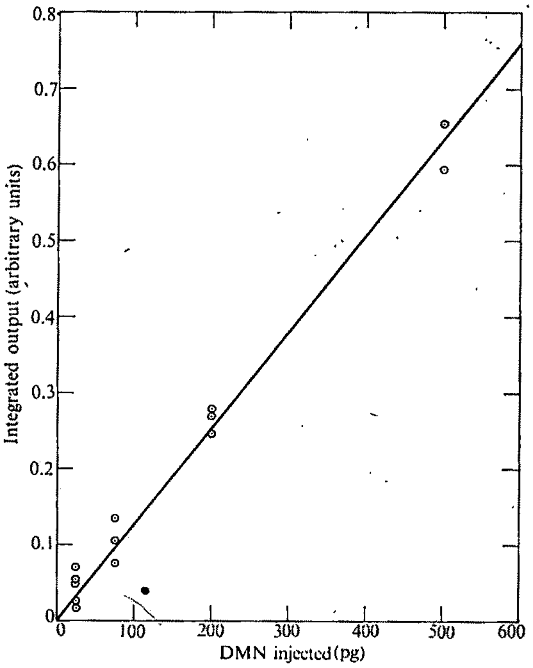

Fig. 1 Plot of integrated instrument response in arbitary units versus picograms of DMN injected in a 5 μl sample.

carrier gas into a catalytic pyrolysis chamber. The activated tungsten oxide catalyst selectively cleaves the N-nitroso compounds. Pyrolysis products and solvent then enter a reaction chamber, maintained at reduced pressure (3.5 torr) by a rotary vacuum pump. Ozone, also enters the reaction chamber, where it reacts with nitric oxide, giving excited nitrogen dioxide. The excited nitrogen dioxide rapidly decays back to its ground state with emission of light in the near infrared (0.6 to 2.8 μm). Using an infrared-sensitive photomultiplier tube, we detect and quantitatively measure the intensity of the emission. A red cut-off filter is used to prevent the photomultiplier tube from responding to emissions at wavelengths shorter than 0.6 μm. The output of the photomultiplier tube is amplified, the intensity of the light emission being directly proportional to the number of mol of N-nitroso compound introduced into the instrument.

A typical calibration curve for DMN is shown in Fig. 1 as a plot of instrument response against pg of DMN injected. At a signal to noise ratio of unity, 35 pg of DMN are required for detection, corresponding to a sensitivity limit of 0.4×10^{-12} mol of N-nitroso compound. The calibration remains linear over six orders of magnitude, from pg to μg levels. A similar mol response has been found for a variety of volatile and non-volatile N-nitroso compounds, including ethyl-N-nitrososarcosinate, diethylnitrosamine, dibutylnitrosamine, diphenylnitrosamine, N-nitroso-N-ethylaniline, and phenylnitrosourea. For N-nitroso-methylurea, because of its extraordinary sensitivity to light, we found that most of it had already decomposed during the course of preparing the calibration solutions but the predicted mol response was obtained when the calibration solutions were prepared using blackened ampules.

A wide variety of compounds with structures similar to the N-nitroso compounds have been tested as 1% solutions in DCM for possible positive interference[11]. No false positives, up to the limit of the instrument sensitivity, were detected.

To test the feasibility of using the analyser as a selective detector for N-nitroso compounds in complex mixtures, recovery experiments were carried out at the μg kg^{-1} level with DMN (a volatile N-nitrosamine) and diphenylnitrosamine (a non-volatile nitrosamine). Five grammes of fresh ground beef or fresh herring were homogenised with 5 ml of DCM and transferred into a Teflon-capped centrifuge tube. The homogenised mixture was spiked with a known volume of a solution containing 1 μg ml^{-1} of the N-nitroso compound. The tube was shaken for 30 min and then centrifuged. Five microlitres of the bottom DCM layer was injected directly into the analyser, without further purification or concentration. The recovery for both the volatile and non-volatile N-nitroso compounds was in the range of 75 to 100% (Table 1). As the materials tested contain a vast number of different compounds, and considering that the materials were only extracted once, recoveries of this order are strong evidence that other compounds do not interfere with the detection of N-nitroso compounds by this method.

Table 1 Recovery experiments of DMN, and diphenylnitrosamine from fresh beef and fresh herring at the μg kg^{-1} level.

Sample	Percentage recovery*
39 μg kg^{-1} DMN in fresh beef	80%
110 μg kg^{-1} DMN in fresh beef	77%
242 μg kg^{-1} DMN in fresh beef	87%
20 μg kg^{-1} DMN in fresh herring	85%
60 μg kg^{-1} DMN in fresh herring	90%
120 μg kg^{-1} DMN in fresh herring	97%
60 μg kg^{-1} diphenylnitrosamine in fresh herring	100%
120 μg kg^{-1} diphenylnitrosamine in fresh herring	75%

* Each experiment performed in duplicate.

Because of the demonstrated specificity of the analyser to N-nitroso compounds, an instrument selective to individual N-nitroso compounds would be available if the N-nitroso compounds could be separted from each other before introduction into the analyser. Relatively crude separation would be required, for it is only necessary to separate N-nitroso compounds from each other, and not from the other substances present. Additional studies are currently under way to interface the analyser with GLC.

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Dissociability and tRNA content of monosomes produced by dimethylnitrosamine and starvation

DIMETHYLNITROSAMINE is an hepatotoxic agent which disaggregates liver polysomes into monosomes[1,2]. Previous investigations have suggested that polysome disaggregation results from alkylation and subsequent fragmentation of mRNA in polysomes[3], producing complexed ribosomes. But, fragmentation has been ruled out by the findings that monosomes produced by dimethylnitrosamine dissociate into subunits in solutions containing high concentrations of KCl[4,5] and that pretreatment of animals with cycloheximide prevents polysome disaggregation by dimethylnitrosamine[6]. In bacteria the single ribosome population contains both complexed and runoff ribosomes which can be distinguished by their dissociability[7] and tRNA content[8]. Therefore, we have compared these properties in monosomes produced by dimethylnitrosamine and by starvation, to determine whether they are runoff ribosomes. Our results show that in spite of their similar dissociability, monosomes produced by dimethylnitrosamine and starvation differ in tRNA content.

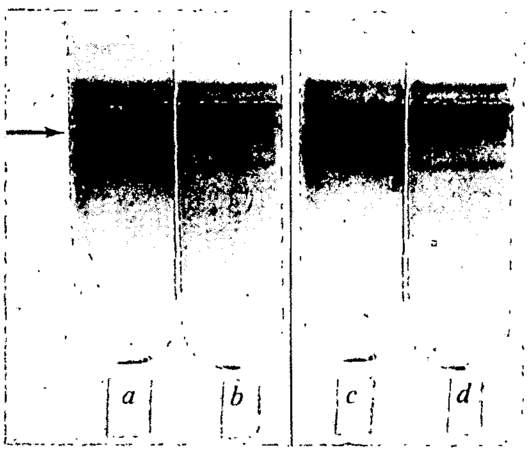

FIG. 1 Ribosome-polysome profiles of mouse livers in photopolymerised linear sucrose gradients. The arrow indicates the position of the 80S band. *a*, One hour after injection of dimethylnitrosamine, 25 mM KCl treatment; *b*, 1 h after injection of dimethylnitrosamine, 0.3 M KCl treatment; *c*, 72 h after starvation, 25 mM KCl treatment; *d*, 72 h after starvation, 0.3 M KCl treatment. Swiss albino male mice were either given a single intraperitoneal injection of 50 mg kg^{-1} body weight of dimethylnitrosamine or were starved. One hour after injection or 72 h after starvation livers were removed and ribosome pellets were prepared as previously described[4] except that the post-mitochondrial supernatant was centrifuged at 35,000 r.p.m. in a type 50 rotor through a cushion containing 1 M sucrose in addition to buffer. To determine the dissociability of monosomes the pellets were rinsed twice and resuspended in buffer containing 10 mM Tris-HCl (*p*H 7.4), 5 mM $MgSO_4$, and either 25 mM KCl or 0.3 M KCl to a final concentration of 50 A_{260} U ml^{-1}. Samples of 0.2 ml were layered onto 0.5–1.2 M linear sucrose gradients containing acrylamide gel components[4]. Gradients were centrifuged at 35,000 r.p.m. in a Spinco Model L centrifuge with a SW 50.1 rotor for 90 min at 5° C and then photopolymerised, sliced, and stained[4,14].

A single dose of dimethylnitrosamine (Fig. 1*a*) or 72 h of starvation (Fig. 1*c*) completely disaggregated liver polysomes into 80S monosomes which dissociated into 40S and 60S subunits in 0.3 M KCl (Fig. 1*b* and *d*). To characterise these ribosomes further, RNA extracted from monosomes was analysed by polyacrylamide gel electrophoresis and the ratios of 4S to 5S RNA were determined. Figure 2 shows a typical profile of RNA extracted from normal mouse liver polysomes (Fig. 2*a* and *b*), monosomes produced by dimethylnitrosamine (Fig. 2*c* and *d*), and monosomes produced by starvation (Fig. 2*e* and *f*). Under these conditions 18S and 28S RNA remained at the top of the gel whereas 4S and 5S RNA readily entered the gel. The 4S peak from normal polysomes was larger in samples in which RNA was extracted after incubation of polysomes with Pronase (Fig. 2*a*) than in samples in which RNA was extracted without previous incubation with Pronase (Fig. 2*b*). Kabat has demonstrated that a portion of the tRNA in polysomes carries nascent peptide chains and that this peptidyl-tRNA is extractable only after detachment of nascent peptide[9]. Therefore, all species of tRNA bound to ribosomes (peptidyl-tRNA, aminoacyl-tRNA and tRNA) are detected in samples previously incubated with Pronase, whereas only tRNA lacking peptide is detected in samples extracted with-

TABLE 1 No. of tRNA molecules on polysomal and single ribosomes

	4S:5S RNA ratio extracted with Pronase	4S:5S RNA ratio extracted without Pronase
Normal polysomal ribosomes	1.7	0.9
Dimethylnitrosamine monosomes	1.7	0.9
Starved monosomes	0.7	0.7
Dimethylnitrosamine monosomes (1.5 mM $MgSO_4$)	1.6	0.7
40S subunit from dimethylnitrosamine monosomes	0	—
60S subunit from dimethylnitrosamine monosomes	1.4	—
Starved monosomes with normal supernatant	0.7	0.7
Dimethylnitrosamine monosomes in 1 M Tris (pH9) for 4 h	1.7	1.4
Dimethylnitrosamine monosomes in 1 M Tris (pH9) for 8 h	1.7	1.6
Dimethylnitrosamine monosomes in 1 M NH_2OH for 1 h	1.7	1.4
Dimethylnitrosamine monosomes in 1 M NH_2OH for 2 h	1.7	1.5
Dimethylnitrosamine monosomes in 1 M NH_2OH for 4 h	1.7	1.7
Dimethylnitrosamine monosomes with puromycin	1.5	1.4

Since each ribosome contains one molecule of 5S RNA the number of tRNA molecules per ribosome was determined from the molar ratio of 4S to 5S RNA[9]. Monosome and polysome pellets were prepared in buffer containing 3 mM $MgSO_4$ unless otherwise indicated. RNA was extracted from these pellets and analysed by electrophoresis as described in Fig. 2. Each peak was weighed and the 4S to 5S molar ratio was calculated by multiplying the value of the 4S peak by 1.5 and dividing by the value of the 5S peak[9]. RNA was extracted from isolated subunits obtained by dissociation of monosomes in 0.3 M KCl and centrifugation in sucrose gradients containing 0.3 M KCl for 4 h at 35,000 r.p.m. in a SW 50.1 rotor. Gradients were scanned at 260 nm and the 40S and 60S peaks were collected. Ribosome subunits were precipitated by the method of Dessev and Grancharov[18]. In some cases monosome pellets from livers treated with dimethynitrosamine were resuspended in 1 M Tris-HCl (*p*H 9) or in 1 M hydroxylamine (*p*H 7)[11]. Sodium dodecyl sulphate (6 mg ml^{-1}) was added and the samples were incubated at 30° C for the time indicated. Other ribosome pellets were resuspended in 50 mM Tris-HCl (*p*H 7.4), 0.5 M KCl, and 3 mM $MgSO_4$ and puromycin-HCl was added to a concentration of 1 mM. The samples were incubated for 30 min in ice and for 20 min at 35° C (ref. 12). RNA was extracted directly from the incubation mixtures. Postribosomal supernatant was prepared by centrifuging postmitochondrial supernatant from normal liver prepared in 50 mM Tris-HCl (*p*H 7.4), 100 mM KCl, and 3 mM $MgSO_4$ at 35,000 r.p.m. for 90 min in a type 50 rotor.

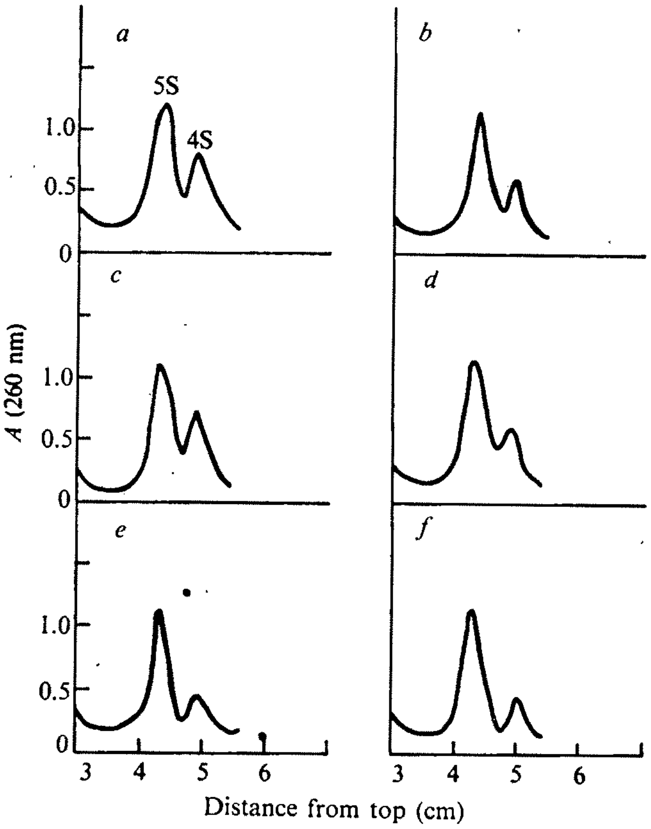

FIG. 2 Electrophoresis of RNA extracted from polysomes and monosomes. The peaks indicate the position of 5S and 4S RNA in the acrylamide gel as measured from the top of the gel. *a*, Normal polysomes with Pronase treatment; *b*, normal polysomes without Pronase treatment; *c*, monosomes 1 h after injection of dimethylnitrosamine with Pronase treatment; *d*, monosomes 1 h after injection of dimethylnitrosamine without Pronase treatment; *e*, monosomes 72 h after starvation with Pronase treatment; *f*, monosomes 72 h after starvation without Pronase treatment. Livers were homogenised in 50 mM Tris-HCl (*p*H 7.4), 100 mM KCl, and 3 mM MgSO$_4$ and 0.2 ml of postmitochondrial supernatant was centrifuged for 90 min in 0.5–1.2 M linear sucrose gradients in the same buffer. Gradients were scanned at 260 nm[15]. The remainder of the postmitochondrial supernatant was centrifuged through a 1 M sucrose cushion in the same buffer. RNA was extracted from ribosome pellets by the chloroform-isoamyl alcohol method of Oda and Joklik[16]. In samples from dimethylnitrosamine-treated and starved animals RNA was extracted only from monosome pellets in which gradient centrifugation revealed complete disaggregation of polysomes into monosomes. Microscopy of nucleolar segregation was also used to ascertain the toxic effect of dimethylnitrosamine[17]. Monosome pellets were resuspended in 5 mM Tris-HCl (*p*H 7.4) and the suspension was extracted immediately or was incubated for 1 h at 30° C with 3 mg ml^{-1} Pronase and 4 mg ml^{-1} sodium dodecyl sulphate[9]. RNA was precipitated overnight at −18° C with two volumes of ethanol and 0. 2 M NaCl. RNA was analysed by electrophoresis in 4% acrylamide gels at 5 mA per gel for 65 min in 36 mM Tris-phosphate (*p*H 7.8), 30 mM Na$_2$HPO$_4$, and 1 mM EDTA as described by Kabat[9]. Gels were scanned at 260 nm by a Gilford spectrophotometer with a linear transporter. RNA (350 ug) was applied to each gel. *E. coli* tRNA was used to estimate the position of the 4S and 5S RNA peaks.

out previous proteolysis. Figure 2 further demonstrates that the RNA profile from monosomes produced by dimethylnitrosamine resembles the profile from normal polysomes. The 4S to 5S RNA ratio is substantially increased on Pronase preincubation (Fig. 2*c* and *d*). Monosomes from starved liver contained a smaller quantity of tRNA (Fig. 2*e* and *f*). Measurement of the 4S and 5S peaks from samples which had been treated with Pronase before extraction yielded about 1.7 tRNA molecules per ribosome for normal polysomes and monosomes produced by dimethylnitrosamine but only 0.7 tRNA molecule per ribosome for monosomes produced by starvation (Table 1). However, polysomes and monosomes produced by dimethylnitrosamine contained only 0.9 molecule of tRNA in samples extracted without Pronase treatment. The ratio of 4S to 5S RNA for monosomes produced by starvation did not change. Thus, polysomes and monosomes produced by dimethylnitrosamine contain approximately 0.8 molecule of peptidyl-tRNA and 0.9 molecule of tRNA or aminoacyl-tRNA. Cornwell[10] has reported that normal liver polysomes contain 1.5 tRNA molecules per ribosome.

To discount the possibility that the tRNA on monosomes produced by dimethylnitrosamine was the result of non-specific adsorption of tRNA to ribosomes, monosomes were prepared in buffer containing 1.5 mM MgSO$_4$. (Davis demonstrated that buffer containing a low concentration of magnesium reduces the amount of non-specifically bound tRNA[8].) Under these conditions monosomes dissociated into 40S and 60S subunits but still retained 1.7 and 0.7 tRNA molecules per ribosome in samples treated with and without Pronase, respectively. Extraction of RNA from isolated subunits revealed that tRNA remained bound to the 60S subunit after dissociation (Table 1). Furthermore, when monosomes produced by starvation were mixed with post-ribosomal supernatant from normal liver and centrifuged through 1 M sucrose in buffer containing 3 mM MgSO$_4$ the ribosomes still contained only 0.7 tRNA molecule per ribosome both with and without Pronase treatment (Table 1). Therefore, the tRNA present on monosomes is probably not due to non-specific binding of tRNA.

If the difference in the ratios of 4S to 5S RNA with and without proteolysis was due to the presence of peptidyl-tRNA on ribosomes, then exposure of ribosomes to conditions which promote cleavage of nascent peptide from peptidyl-tRNA should have given the same ratio of 4S to 5S RNA whether or not the sample was incubated with Pronase before extraction. Table 1 shows that the difference between ratios in samples extracted with and without Pronase decreased as monosomes produced by dimethylnitrosamine were incubated at 30° C for increasing periods in the presence of 1 M Tris-HCl (*p*H 9) or 1 M hydroxylamine (*p*H 7). The ratios were nearly identical after incubation in Tris-HCl for 8 h or in hydroxylamine for 4 h (Table 1). These conditions enhance cleavage of nascent peptide from peptidyl-tRNA[11]. Similarly, incubation of monosomes produced by dimethylnitrosamine with 1 mM puromycin at high ionic strength according to Blobel and Sabatini[12] eliminated the difference between ratios of 4S to 5S RNA extracted with and without Pronase treatment (Table 1).

Therefore, the monosomes which accumulate during starvation are likely to be runoff ribosomes since they dissociate into subunits and are free of peptidyl-tRNA[7–9]. The monosomes which accumulate after a single dose of dimethylnitrosamine, however, are unlikely to be runoff ribosomes or complexed ribosomes since they dissociate into subunits like runoff ribosomes, but contain peptidyl-tRNA and tRNA (aminoacylated and free tRNA) like complexed ribosomes. Complexed ribosomes do not dissociate into subunits under these ionic conditions[4]. Other investigators have demonstrated that only ribosomes free of mRNA dissociate into subunits, whereas ribosomes still attached to mRNA do not dissociate even if stripped of nascent peptide[13]. Therefore, the difference in dissociability between complexed ribosomes and monosomes produced by dimethylnitrosamine suggests that the latter monosomes are free of mRNA. These results suggest that dimethylnitrosamine disaggregates liver polysomes by promoting the premature fall off of ribosomes from mRNA during elongation.

Although the mechanism of polysome disaggregation by dimethylnitrosamine has not been ascertained, this study suggests that *in vivo* it is not fragmentation of mRNA or inhibition of initiation.

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Multiple forms of SS → DS RNA polymerase activity in reovirus-infected cells

The genome of reovirus consists of a unique set of ten segments of double-stranded (DS) RNA[1-3]. This DS RNA is replicated in infected cells on a preformed single-stranded (SS) RNA template which has the same polarity as messenger RNA[4-7].

To gain insight into the mechanism by which the 10 DS RNA segments are assembled and encapsidated during viral morphogenesis we are studying the properties of the enzymes responsible for the synthesis of DS RNA in infected cells. Recently we described the isolation of a SS RNA-dependent DS RNA polymerase (SS → DS RNA polymerase) from infected L cells which is associated with virion-like particles[8]. Since the DS RNA synthesised by these particles remains associated with them and since DS RNA is synthesised on a preformed SS RNA template, we suggested that the ten segments of SS RNA associate with viral proteins in a virion-like particle and that DS RNA is synthesised within these particles[8].

We have extended these studies and have identified several classes of subviral particles which possess SS → DS RNA polymerase activity. Each catalyses the synthesis of different size classes of DS RNA segments.

A cytoplasmic extract was prepared from infected cells maintained at 31° C and collected 18–20 h after infection, when RNA synthesis is at a maximum. All SS → DS RNA polymerase activity in this extract was pelleted by centrifugation for 3 h at 35,000 r.p.m. When the material in this pellet was centrifuged in a glycerol gradient and the fractions assayed for SS → DS RNA polymerase activity, a broad spectrum of polymerase activity was observed (Fig. 1). Reproducibly, two peaks of polymerase activity were found which correspond to structures with approximate sedimentation coefficients of 550 and 250S in comparison with virions (630S) and top component (470S). The major polymerase peak always sedimented at 250S. Approximately 20% of the polymerase activity was recovered in the pellet of the gradient. No loss in polymerase activity was observed after gradient centrifugation, when compared with the enzyme activity in the cytoplasmic pellet before centrifugation.

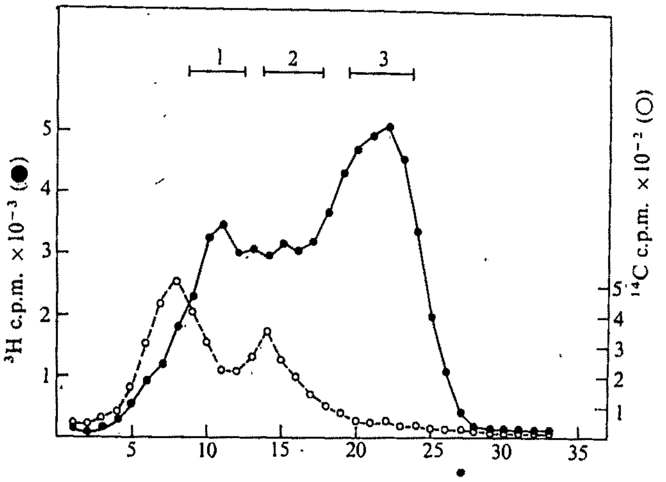

Fig. 1 Distribution of SS → DS RNA polymerases in glycerol-density gradients. 2×10^8 infected spinner cells maintained at 31° C and collected 18 h post infection[7], were resuspended in 5 ml 0.01 M NaCl-0.1 M Tris, *p*H 8.0 (S-T buffer) and dounced in the presence of 1% Triton X-100 and 1% DOC. Nuclei were removed by centrifugation at 1,500 r.p.m. for 5 min. All procedures were performed at 4° C. The supernatant was layered over a 4 ml glycerol cushion (40% in 10^{-3}M Tris, *p*H 8.0) and centrifuged in a SW 41 rotor for 3 h at 38,000 r.p.m. The pellet was resuspended by sonication in 0.7 ml S-T buffer mixed with a small sample of ^{14}C-labelled purified reovirions and top component, and layered onto a 20–40% glycerol (in 10^{-3}M Tris, *p*H 8.0) gradient. The gradient was centrifuged at 30,000 r.p.m. for 1 h in a SW 41 rotor. Fractions were collected, diluted with an equal volume of 2 × S-T buffer and 0.25 ml of each fraction was assayed for SS→DS RNA polymerase activity by the addition of 0.1 μmol each of ATP, GTP, and CTP, 6×10^{-3} μmol UTP, 5μCi 3H-UTP (15 Ci-mmol^{-1}), 0.9 μmol $MgCl_2$, 0.9 μmol β-mercaptoethanol and 0.1 μg actinomycin D. After incubation for 1 h at 39° C, 0.2 μmol cold UTP was added. After addition of NaCl to a final concentration of 0.15 M and 20 μg-ml^{-1} pancreatic ribonuclease, samples were incubated at 37° C for 30 min. The samples were precipitated with an equal volume of 10% trichloroacetic acid and the precipitate collected on Millipore filters. Radioactivity was determined by liquid scintillation counting[8]. Sedimentation is from the right to the left in the Figure. 22% of the total polymerase activity was recovered in the pellet of the gradient. The sedimentation coefficients of purified virions and top component are 630 S and 470 S respectively. ●——●, SS → DS RNA polymerase products; ○– – –○, ^{14}C virion and top component.

To investigate the properties of the SS → DS RNA polymerase activities, fractions were combined as shown in Fig. 1 and designated DS RNA polymerase 1, 2 and 3 respectively. The band of enzyme activity sedimenting at approximately 550S (DS RNA polymerase 1) is the enzyme described previously[8].

The DS RNA products of each polymerase were analysed on sucrose density gradients and polyacrylamide gels to determine whether the three polymerases synthesise all ten species of DS RNA. Viral DS RNA is separated into three size classes, L, M and S, in sucrose-density gradients[1,2]. As shown in Fig. 2, the three SS→DS RNA polymerases synthesise the three size classes to different extents. DS RNA polymerase 1 synthesises predominantly DS RNA of the L class (Fig. 2*a*), DS RNA polymerase 2 synthesises the M class in excess over L and S DS RNA (Fig. 2*b*), and DS RNA polymerase 3 predominantly the S class (Fig. 2*c*).

Further separation of the viral DS RNA into ten species is obtained by polyacrylamide gel electrophoresis[3] (Fig. 3*d*). DS RNA polymerase 1 synthesises all ten species of DS RNA, but the predominant species are the three L species (Fig. 3*a*). Again DS RNA polymerase 2 synthesises the three M species in relative excess (Fig. 3*b*) and DS RNA polymerase 3 synthesises a relative excess of the four S species (Fig. 3*c*).

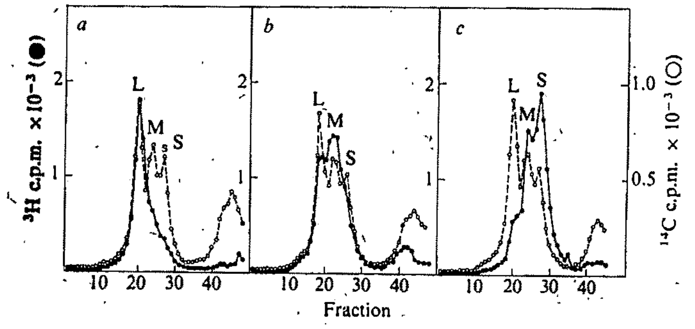

Fig. 2 Sedimentation of the DS RNA products of the three SS → DS RNA polymerases in sucrose-LiDS gradients. DS RNA polymerases 1, 2, and 3 were isolated from a glycerol gradient by combining fractions as described in Fig. 1 and incubated with the components of the polymerase reaction mixture. After the reaction, RNA was extracted with phenol, precipitated with ethanol and resuspended in 2 × SSC. After the addition of ^{14}C-DS RNA extracted from purified virions[9], the mixture was incubated for 15 min at 37° C with 5 μg ml^{-1} pancreatic RNase[8]. After the addition of LiDS to 1%, the sample was layered on a 15–30% sucrose-LiDS gradient and centrifuged in a SW 27 rotor for 24 h at 25,000 r.p.m. Fractions were collected and precipitated with an equal volume of 30% trichloroacetic acid in the presence of bovine serum albumin (10 μg ml^{-1}). Sedimentation is from right to left, *a*, *b*, and *c* represent the products of SS → DS RNA polymerases 1, 2, and 3 respectively. ●——●, ^{3}H-labelled SS →DS RNA polymerase products; o – – o, ^{14}C-DS RNA extracted from purified virions.

In virions, the ten DS RNA segments are surrounded by two protein layers. The outer protein layer can be removed by chymotryptic digestion to produce a core which contains the genome and the inner capsid proteins. This structure has a buoyant density in CsCl of 1.43 g cm^{-3} as compared with 1.36 g cm^{-3} for complete virions[9].

We have suggested previously that DS RNA polymerase 1 consists of a core-like structure to the outside of which additional protein molecules are attached and that DS RNA is synthesised within this particle[8]. The evidence for this is as follows: the enzyme sediments slightly slower than virions in sucrose-density gradients, its buoyant density is very close to that of virions (1.34 g cm^{-3} compared with 1.36 g cm^{-3} for virions) and after chymotrypsin treatment its density increases to that of viral cores (1.43 g cm^{-3}). Furthermore, DS RNA synthesised by these particles is not released but remains associated with structures which have the same sedimentation properties in sucrose and caesium gradients as the enzyme itself.

Similar experiments were undertaken to determine whether DS RNA polymerases 2 and 3 are also associated with subviral particles. DS RNA polymerases 2 and 3 resemble DS RNA polymerase 1 in that their buoyant densities are also 1.34 g cm^{-3}. In the case of DS RNA polymerase 3, a second but minor enzymatic activity was sometimes detected which had an approximate buoyant density of 1.43 g cm^{-3}. It was not possible to determine the effect of chymotrypsin treatment on the densities of DS RNA polymerases 2 and 3, since both lost their enzymatic activity after chymotrypsin treatment.

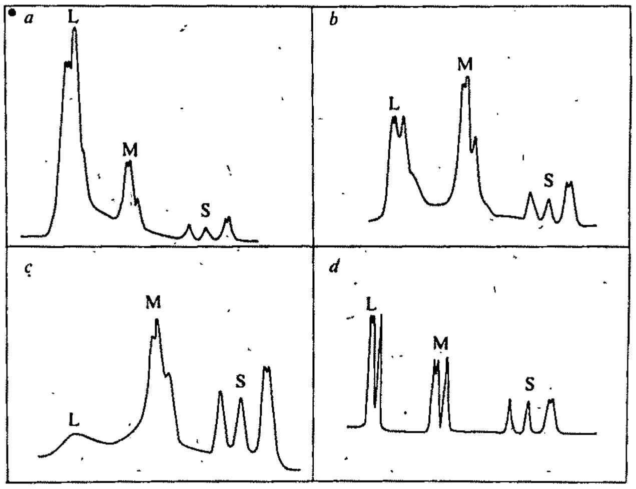

Fig. 3 Polyacrylamide-gel electrophoresis of the DS RNA products of DS RNA polymerases 1, 2, and 3. DS RNA polymerases 1, 2, and 3 were incubated under standard reaction conditions except that ^{3}H-UTP was replaced by ^{32}P-UTP (10 μCi per 0.25 ml). RNA was extracted with phenol, treated with 5 μg RNase in 2 × SSC (see legend to Fig. 2) and electrophoresed on 7.5% polyacrylamide slab gels, as described previously[10]. ^{14}C-labelled DS RNA extracted from purified virions was also resuspended in 2 × SSC and treated with RNase before electrophoresis. Autoradiograms were prepared from these gels and scanned in a Gilford recording spectrophotometer[10]. *a*, *b*, and *c*, products of DS RNA polymerase 1, 2, and 3 respectively; *d*, DS RNA extracted from purified virions.

Similarly, DS RNA polymerases 2 and 3 resemble DS RNA polymerase 1 in that DS RNA synthesised *in vitro* remains associated with the enzyme complexes. In the case of DS RNA polymerase 2, newly made DS RNA is associated with structures with a buoyant density of 1.34 g cm^{-3} which increases to 1.43 g cm^{-3} after chymotrypsin treatment. The DS RNA polymerase 3 product also has a buoyant density of 1.34 g cm^{-3}, but 50 to 75% of these structures are completely degraded by chymotrypsin treatment. Infrequently, the DS RNA polymerase 3 product contained a second particle with a buoyant density of 1.43 g cm^{-3}. This may correspond to particles described by Sakuma and Watanabe[11]. Possibly this is a product of the second particle sometimes found in DS RNA polymerase 3 preparations which also has a density of 1.43 g cm^{-3}.

In view of these findings we propose that ten segments of SS RNA associate with viral proteins to form a particle which can only replicate a limited number of SS RNA species. Before replication of other SS RNA species can occur, certain sequential changes in the structure of such particles are necessary. These changes would result in particles more closely resembling virions as judged by increased resistance of the inner proteins to chymotryptic digestion and altered sedimentation rates. One of the first particles formed is probably DS RNA polymerase 3, since this particle resembles virions the least.

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*p*H, Salinity and Temperature Tolerance of Lake Magadi *Tilapia*

THERE are several species of *Tilapia* isolated in the alkaline lakes of the Great Rift Valley of Africa, living in extreme conditions of temperature, salinity and *p*H. One of these fish, the Lake Magadi *Tilapia* (*Tilapia grahami*), lives in the lagoons and alkaline volcanic springs around the margin of Lake Magadi in Kenya[1]. The main salts of the springs, and the lake, are carbonate and bicarbonate of sodium.

To continue with studies on osmoregulation and acid-base balance in *Tilapia grahami*, we made some observations on its tolerance to changes in *p*H, salinity and temperature. Each of the three series of experiments was carried out on different fish as were the individual experiments within each series. Each experiment involved four to six medium-sized fish 3 to 5 cm long, which were kept in a glass jar. The fish were caught in nets in a lagoon (Fish Springs[1]) and were transported to Nairobi in water taken from the same lagoon, which had *p*H values of 9.8 to 10.1, osmolarity of 518 to 600 mOsm per kg H_2O and a temperature, measured on different days, of 35.2 to 38.0° C.

In Nairobi, some fish were transferred to tap water, which was well tolerated. Others were transferred to dilute solutions of hydrochloric acid of *p*H 3 to 6 or sodium hydroxide (of *p*H 8 to 12) in tap water. Those exposed to a *p*H of 3 to 4 or a *p*H of 12 died within 2 to 6 h. Fish kept at *p*H values between 5 and 11 showed no ill effects after 24 h. Throughout the experimental period the *p*H was regularly measured and adjusted by adding acid or base. Most experiments of this type were performed at 22° to 23° C, but similar *p*H tolerance limits were found in a few fish studied at 35° to 36° C during the first day after they had been brought from the lagoon.

Tolerance to salinity changes was tested at 22° to 23° C in 2 to 6% solutions of sodium chloride in tap water. Fish kept in 2 to 3% solutions were apparently in good condition after 24 h, those in 4% solution were alive after 8 to 10 h but died soon afterwards. Those maintained in greater concentrations died within a few hours.

Temperature tolerance was tested in fish kept in water from the lagoon. At 10° to 12° C the fish died within 1 to 2 h, but they survived after 24 h at 16° C. Temperatures of 22° to 23° C were well tolerated for several days. The upper limit for temperatures tolerated for at least 24 h seemed to be about 40° C. If the fish had been kept at 22° to 23° C for some days, however, immediate transfer to water of moderately high temperatures (35° to 36° C) was lethal.

Our study indicates that the ability of *T. grahami* to survive in alkaline waters is superior to that known for any other fish, while acid waters (*p*H 5) and salinity changes have been shown to be equally well tolerated by adult individuals of several freshwater and euryhaline species[2]. The range of thermal tolerance of *T. grahami* is less than that previously demonstrated in the desert pupfish, *Cyprinodon*[3], but the upper lethal temperatures are about the same. Previous observations have indicated that individuals of *T. grahami*, particularly brooding females, during their movements between the different pools may survive transient exposure to water temperatures of 40° to 44° C (compare refs 1 and 4).

In addition to high *p*H, salinity and temperature, the Lake Magadi *Tilapia* seems to be faced with low levels of dissolved oxygen. Preliminary measurements, obtained with the azide modification of the iodometric method[5], showed oxygen levels of 1.1, 2.2 and 3.3 mg l^{-1} in water from outlets of hot spring, main lagoon and shallow pool, respectively. Fish were observed in all these locations. Those used in our experiments were caught in the main lagoon; large individuals seemed to prefer the more shallow pools. Large amounts of free carbon dioxide also seem to be well tolerated as the fish showed no ill effects when kept in water from the lagoon while the water was acidified to *p*H 5 by dropwise addition of hydrochloric acid. Addition of strong acids to waters with a high bicarbonate content can produce concentrations of carbon dioxide which are fatal to other species of fish[2].

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IQ and Race

JENSEN has argued that the undoubted differences in mean IQ scores between racial groups in the United States reflect differences in genetic endowment. But a major problem in the interpretation of these differences is the difficulty of studying environmental and genetic effects separately. Each race tends to have, for historical reasons, characteristic child-rearing practices which are to some extent independent of social class. Hence the argument that because black children tend to have lower IQs than white children of the same social class they are genetically inferior is unconvincing; the inferior test scores could result from differences in the microenvironment of the family, such as linguistic practices and attitudes to achievement.

My colleagues and I have recently had an opportunity to study children of different racial origins reared in an identical environment. These were illegitimate children admitted to residential nurseries as infants until such time as their mothers could look after them or they could be found adoptive homes. All those tested had been healthy, full-term babies: the residential nurseries, run by three voluntary societies, were in many respects of high quality, with a generous staff–child ratio and a plentiful supply of toys and books. Because the nurseries tended to be isolated the children did not meet community colour prejudice, and within the nurseries we saw no sign of such prejudice.

Table 1 Test Scores of Eighty-five Residential Nursery Children

	Reynell comprehension*			Reynell expression*			Minnesota non-verbal†		
	N	Mean	s.d.	*N*	Mean	s.d.	*N*	Mean	s.d.
White children	39	102.6	10.0	39	98.5	11.2	24	101.3	11.1
Black children‡	22	106.9	8.7	22	97.8	8.5	15	105.7	7.4
Mixed race children§	24	105.7	10.2	24	99.3	8.7	15	109.8	7.2
White against non-white		t=1.70, n.s.			t=0.02, n.s.			t=2.51, $P<0.02$	

* Converted to a mean of 100, s.d. 10.
† Only given to children aged 3 yr and older.
‡ Children with two West Indian or African parents.
§ Children with one West Indian or African parent and one white parent.

Table 2 Wechsler Pre-school and Primary Scale of Intelligence Full Scale IQs of Sixty-four Children Aged 4.5 yr

	Institutional			Adopted			Restored to mother		
	N	Mean	s.d.	*N*	Mean	s.d.	*N*	Mean	s.d.
White children	10	101.2	10.7	17	113.0	7.8	9	98.2	10.8
Mixed race children	7	109.3	8.2	7	119.9	16.6	5	102.2	12.3
Black children	8	105.6	10.3	0			1	106.0	
White against non-white		$t=1.55$, n.s.			$t=1.45$, n.s.			$t=0.80$, n.s.	

In the first study we tested all the healthy children in thirteen residential nursery groups who had been there at least six months and who were aged 2 yr 0 month–4 yr 11 months. Thirty-nine children had two white parents, twenty-two had two West Indian or African parents, and twenty-four were racially mixed. Seventy per cent had been admitted before their first birthday, and 86% before their second[1]. The children were tested with a non-verbal intelligence test and a test of language comprehension and expression. Table 1 shows that the mean test scores of the different racial groups were very similar, and the only significant difference was in favour of the non-white children.

In the second study we tested all the available children aged 4.5 yr who had been admitted to any of the residential nurseries of these voluntary societies by the age of 4 months and remained there until at least the age of 2 yr. We found sixty-four such children: twenty-five were still in institutions, twenty-four had been adopted into white families at a mean age of 3.1 yr, and fifteen had been restored to their mothers (all but one of whom were white) at a mean age of 3.5 yr[2]. Table 2 shows that the mean IQs of the different racial groups in each environment were very similar.

These studies were not undertaken with the aim of comparing the scores of different racial groups, but rather to examine the effects of different environments on development. In the first study children in nurseries where the quality of the staff talk to children (as assessed in observations) was highest had mean language comprehension scores one and a half standard deviations above those of children in the nurseries where the quality of staff talk was lowest. In the second study the adopted children had significantly higher mean IQs than either the children still in nurseries or those restored to their mothers, and these differences were related to measured aspects of the children's environment.

In both studies genetic aspects were neither controlled nor adequately known, that is, the children had not been randomly assigned to different environments and no parental IQs were available. It could therefore be argued that the non-white parents may have been of higher IQ than the white parents, or that children of higher IQ parents had been adopted or placed in nurseries where the staff talk was of high quality. We found no evidence to support such hypotheses. Children were placed in whichever nursery had a vacancy, and no attempt was made to assess the IQ of the children or their natural parents before placing them for adoption. The occupation of a third of the natural fathers was unknown, but for the rest there was no significant difference between the proportions of manual and non-manual workers in the different racial groups or different home or nursery environments. On the other hand, in both studies the relationship between the children's test scores and measured aspects of the environment was shown to be large and significant.

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book reviews

Intuitive science

Thematic Origins of Scientific Thought: Kepler to Einstein. By Gerald Holton. Pp. 495. (Harvard University: Cambridge, September 1973.) $10 cloth; $3.95 paper.

THE fifteen papers in this collection, all published previously in a variety of books and journals over the last twenty years, are printed here largely in their original form, but with the addition of a short general introduction to explain why they are all brought together into one volume. Each deals in some way with what Professor Holton terms the "themata" of science, that is the "unverifiable, unfalsifiable, and yet not-quite-arbitrary hypotheses" which are based not on empirical experiment and observation, nor on logical deduction, but simply on our general intuitions and beliefs about how we expect nature to behave.

The first three essays attempt to explain the concept of themata at length; the second essay concentrates on the metaphysical ideas underlying Kepler's description of planetary motions, and uses these as an illustration of how such ideas—in Kepler's work the belief that the Sun must be the centre of the Universe, and the belief in the underlying mathematical harmony of nature—form an essential part of the processes of scientific discovery. This is the only essay in which Professor Holton makes a lengthy excursion into early science; elsewhere his examples are drawn mainly from modern physics.

The second section, which comprises well over a third of the book, is devoted to relativity theory. The papers here, still very much concerned with the general question of underlying themata, cover the field of Professor Holton's most thorough historical research, and reflect his extensive study of the material in the Einstein Archive at Princeton Institute for Advanced Study. In particular the ninth essay shows, by extensive reference to source material, that Einstein received the inspiration for his relativity theory not so much from the famous Michelson-Morley interferometer experiments (as tradition would have it) as from the thematic influences from his contemporaries and from his own view of nature.

The next three essays are devoted to a discussion of how the themata of science (here referred to as the "non-rational substructure") are becoming increasingly less acceptable as a facet of formal science. A distinction is drawn between "private science" and "public science"—the former the unrecorded progress of the individual towards a new theory, and the latter its presentation to the outside world, stripped of all description of the initial gropings of its author and set down tidily and rationally. The remaining two essays in the book deal with the role of science in education; Professor Holton asserts here that it is both essential and possible to give the man in the street, or at least the schoolboy at his desk, an adequate awareness of the present state of science, an awareness necessary to the health of our technologically based society. We must not only look outside science for some of the themata which govern scientific discovery, but must also allow science itself to operate in a reciprocal fashion upon our other fields of understanding. To achieve this, there must be a radical redesign of school and college curricula.

All the essays are fascinating and thought provoking (in his introduction Professor Holton writes "a chief aim of these essays is to raise new questions for research rather than merely to answer old ones"), and they are written for the most part in a language accessible to scientist and historian alike, despite the fact that many of the topics treated are by no means easy to present briefly and clearly to the uninformed reader. But there is some clumsiness in his exposition of the meaning of the term "themata" both in the introduction and in the first essay; his use of a three dimensional "space", with x, y and z directions representing respectively the empirical, logical and thematic components of scientific propositions seems to me both unnecessary and confusing. The concept of themata is not so complicated as to warrant such a laboured model for its explanation.

There are two difficulties inherent in an unedited collection of papers of this sort. First, there is inevitable overlapping between the papers, and here this is particularly so in the section on relativity theory; it is tempting to wish that the papers had been integrated into one coherent whole. Second, although the title of the book implies a common thread which runs through all the papers, many of them were not written with the primary or single purpose of describing the "thematic origins of scientific thought". Thus the twelfth essay, *Models for Understanding the Growth of Research*, is more a study of the rapid increase in scientific information and investigation in recent decades and its general causes than a specific study of underlying themata currently guiding research.

The study of themata is by no means new in the history and philosophy of science (although this name for them is a little unfamiliar); and many of these papers were written so long ago that they have lost some of their original freshness—the author readily admits this. But for the scientist coming newly to the history of his subject, or for the layman seeking some insight into the nature of research, this collection of papers should prove enlightening, and convince him of the essential humanity of science.

LAURA TILLING

Disease distribution

Epidemiology of Neurologic and Sense Organ Disorders. By Leonard T. Kurland, John F. Kurtzke, and Irving D. Goldberg. Pp. xxx+436. (Harvard University: Cambridge; Oxford University: London, September 1973.) £7.

THIS monograph is one of a series of reviews of the epidemiology of various conditions published by the American Public Health Association. The series fills a long-standing need for material of this type. This particular book reviews morbidity and mortality data—a number of the others have confined themselves to the latter. The title, "Neurologic and Sense Organ Disorders", is slightly misleading because there is no reference to cerebrovascular accidents, cerebral tumours or cerebral infectious conditions. The volume is otherwise comprehensive.

The first chapter contains an introduction to epidemiology. This is inappropriate: it is too short to do justice to the subject and does not describe it well. Further chapters are devoted to convulsive disorders, Parkinsonism, multiple sclerosis, amyotrophic lateral sclerosis and other motor neurone disorders, muscular dystrophy and other myopathies, myasthenia gravis, Down's syndrome, congenital malformations of the nervous system and other specific neurological disorders, cerebral palsy, blindness and hearing disorders.

The first seven chapters, particularly those concerned with multiple sclerosis, amyotrophic lateral sclerosis and muscular dystrophy, provide superb analyses of mortality and morbidity information as well as reviewing the aetiology of these conditions. The authors do not perhaps deal quite as well as they should with the problems of mortality certification in multiple sclerosis but they give one of the best descriptions I have read of the importance of epidemiological studies of migration in disentangling possible cause. The studies on epilepsy and other convulsive disorders give a most comprehensive review of the epidemiology of these conditions and demonstrate the value of mortality and morbidity statistics. There is also a reasonable analysis of diagnostic and other problems in this context.

The latter chapters are disappointing and highlight the difficulty of writing definitive works on such a wide variety of conditions. The chapters on Down's syndrome, congenital malformations and facial nerve palsy make no mention of possible aetiological factors and do not attempt to discuss the ways in which these conditions may arise. In the review of Down's syndrome, for example, nothing is said about the maternal age factor, the difference in aetiology between Down's syndrome in the offspring of young and old mothers, nor about the association between Down's syndrome and leukaemia.

With these reservations, the book is outstanding. WALTER W. HOLLAND

Sand surfaces

Atlas of Quartz Sand Surface Textures. By David Krinsley and John C. Doornkamp. (Cambridge Earth Science Series.) Pp. x+91. (Cambridge University: London, July 1973.) £6.50; $19.50.

A METHOD of environmental analysis which depends upon descriptions of grain surface textures seen under the electron microscope from a wide variety of known environments has the inherent disadvantage that it requires a considerable accumulated experience and cannot yield results by a few simple measurements. Thus, the technique has largely been used by those prepared to invest a large amount of time, an investment which has seemed unlikely to be repaid by the results obtained. Krinsley and Doornkamp have now tried to make their experience available to those who might simply wish to use the technique as one of several means of analysis rather than embark upon an ambitious research programme. To do this, they have assembled a series of 122 excellent photographs which they claim to represent the principal associations of textures which, when taken together, could be used to characterise an environment.

Instead of the potential user having to undertake an exhaustive search of the literature to construct for himself a pattern of variation, this is now done for him by the authors. The book also contains an introduction to the transmission and scanning electron microscopes, and their development and use in these studies, the preparation of samples; principles of interpretation; a particularly useful diagram showing the frequency of certain types of structures from a variety of environments; and a complete bibliography of the field.

It is clear that the book cannot be used directly as a key to environment, as it is the associations of textures which are important. The statistical analysis which this implies is not necessarily as easy as it may seem, for the number of clearly differentiated surface features which could be used in such an analysis are few. This leads to the general criticism that although analysis is based on descriptions of surface features, these are often vague and over-generalised, and often difficult to follow, such as the description on page 14 of mechanical V-shaped patterns. For example, although abundant reference is made to upturned plates, this term seems to cover a broad variety of forms which could usefully be differentiated.

For young research workers looking for rewarding topics, the book immediately suggests two. The possibility of quantified analysis; and the experimental reproduction of textures which might enable us to identify the processes at work and thus enable us better to specify the nature of the environment. The authors' own structural interpretations are sometimes unconvincing. But this lucid exposition in such a useful form will persuade many to use a technique which they may have previously regarded with awe or suspicion, and should find its way on to the bookshelves of most sedimentologists.

G. S. BOULTON

Fighting malaria

Malaria: The Design, Use and Mode of Action of Chemotherapeutic Agents. By R. M. Pinder. Pp. 336. (Scientechnica: Bristol, September 1973.) £10.

THE preface of this book states that malaria remains a formidable global problem and that it is still the major killing disease of the tropics. One could not disagree with this statement even though the achievements of malaria eradication in many countries outside the truly tropical belt should not be underestimated. The partial failure of global malaria eradication is not due solely to the resistance of malaria parasites to drugs or to insecticide resistance of Anopheles vectors of the disease. This oversimplified explanation of a complex situation cannot be easily accepted by informed readers.

The book is composed of two unequal and rather unbalanced parts. Out of nine chapters six are devoted to chemotherapy of malaria; they comprise the testing and evaluation of antimalarials; a summary of the present status of various drugs, an outline of newer approaches to malaria chemotherapy, an account of miscellaneous compounds, including many experimental ones, a discussion of the mode of action of antimalarials and the review of the present status of drug resistance in malaria.

It is obvious that the author has had access to a formidable collection of reprints and index cards. And yet I missed in this part of the book an attempt at critical analysis of the laboriously collected information. It is as if anything that has been published were of equal importance just because it appeared in print. This outline of problems of chemotherapy of malaria supported by numerous references is, however, of some value although it cannot match the excellence and authoritativeness of W. Peters's *Chemotherapy and Drug Resistance in Malaria* published in 1970.

The remaining three chapters of the book must have been written as an afterthought, perhaps to justify the all-embracing main title which overshadows the more appropriate subtitle. The chapter dealing with epidemiology of malaria gives a succinct account of the progress of malaria eradication and of its reverses; this is followed by a brief review of entomological and parasitological aspects. A chapter on malaria parasites includes the life cycle, the symptoms of clinical infection, and a notion of immunity. It is here that the author's nodding acquaintance with many aspects of malariology is evident. There are many statements that are either half true or quite wrong. Thus, neither pyrimethamine nor chloroquine has been "massively" used in common salt and the number of projects with medicated salt was small (pages 16–17). *P. falciparum* and *P. vivax* do not occur in higher apes in Africa (page 35). The population of tropical Africa is not infected *in toto* by quartan malaria (page 36). Sulphonamides are not causal prophylactic drugs (page 40).

The last chapter entitled "Malaria, the Way of the Dodo?" has also a quota of errors and infelicities (such as reference to the "wily plasmodium") that would be tedious to quote; however, the possibility of altering the man-biting habit of anopheline mosquito visualised by the author as a method of malaria eradication indicates his fleeting acquaintance with the discipline of malariology and with the realities of malaria control. To a question of for whom this book has been written I can give no satisfactory answer.

L. J. BRUCE-CHWATT

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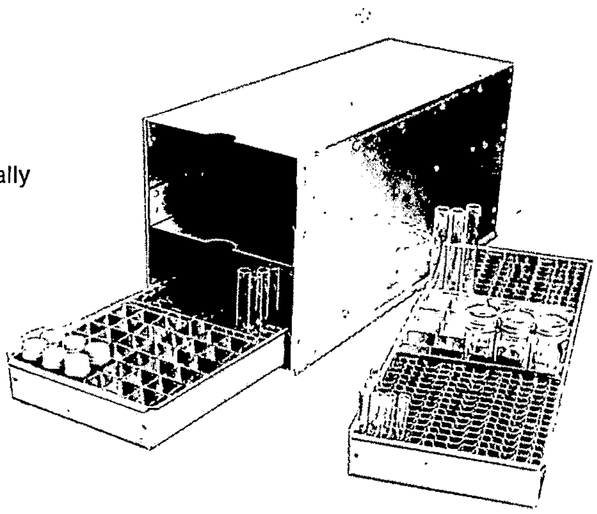

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Applications should be received by the Clerk of the Salters' Company, 36 Portland Place, London W1N 4DL by **March 1, 1974.** Forms of application may be had on request.

No applicant can be appointed without interview. (382)

SYMPOSIUM

INTERNATIONAL SYMPOSIUM ON ARTICULAR Cartilage to be held at Imperial College, London, on September 2-6, 1974. Will consist of invited papers and short communications. Deadline for receipt of abstracts for short communications March 15, 1974.—Further details and registration forms from the Conference Secretary, Biomechanics Unit, Mechanical Engineering Department, Imperial College, London, S.W.7. (387)

CONFERENCE

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

CONFERENCE ON ARTICULAR CARTILAGE

An International Conference on Articular Cartilage is to be held at Imperial College from September 2 to September 6, 1974. Each session of the conference will consist of papers from invited speakers, a number of shorter communications, and general discussion.

The short papers will be selected from abstracts of not more than 300 words, which should reach the organisers by March 15, 1974.

Further details and registration forms from: Articular Cartilage Conference, Biomechanics Unit, Department of Mechanical Engineering, Imperial College of Science and Technology, London, S.W.7. (388)

MISCELLANEOUS

LARGE TELESCOPE USERS

The Science Research Council invites applications to its Large Telescope Users' Panel for observing time as follows:

(a) on the 98-inch Isaac Newton Telescope at Herstmonceux during the three months 1974 July to September inclusive; and

(b) on the 20-inch and 40-inch reflectors at Sutherland during the six months 1974 October 1975 March inclusive.

In considering the allocation of time, the Panel will especially support group applications, particularly where they result from collaboration between a university department and a Royal Observatory.

Applications must be made in respect of proposed programmes that cannot be accomplished on telescopes of smaller aperture, and will be considered by the Panel at its spring meeting. They should be submitted by February 15 on a form obtainable from the Secretary of the Panel, Mr. C. A. Murray, Royal Greenwich Observatory, Herstmonceux Castle, Hailsham, Sussex.

Advance Notice

U.K. observers have been invited to submit applications for observing time on the Anglo-Australian Telescope for the six months beginning 1975 January 1. These will be considered by the Panel at its summer meeting. (391)

FOR SALE AND WANTED

Always wanted:
BACK RUNS OF JOURNALS
IN SCIENCE AND THE HUMANITIES
WM. DAWSON & SONS LTD.
Back Issues Department,
Cannon House, Park Farm Road,
Folkestone, Kent, England.
Tel.: Folkestone 57421. (1)

FIRST ANNOUNCEMENT

NATO Advanced Study Institutes Programme

COURSE: **REGULATION OF FUNCTION AND GROWTH OF EUKARYOTIC CELLS BY INTRACELLULAR CYCLIC NUCLEOTIDES.**

PLACE: WÉPION-NAMUR (BELGIUM).

DATE: SEPTEMBER 23–OCTOBER 3, 1974.

ORGANIZER: J. E. DUMONT (UNIVERSITY OF BRUSSELS).

INFORMATION: MISS CH. BORREY (COURSE),
Institut de Recherche Interdisciplinaire,
School of Medicine,
Boulevard de Waterloo, 115,
B-1000 Brussels (Belgium). (424)

THE QUEEN'S UNIVERSITY OF BELFAST

VISITING STUDENTSHIPS

Applications are invited for Visiting Studentships tenable at the Queen's University of Belfast from October 1, 1974. Candidates should have good honours degrees and have shown aptitude for research.

The studentships are of the value of £700 to £800 per annum for two years with a possible extension for a third year. Up to £100 for initial travelling expenses is available for students coming from outside the British Isles, and up to £25 for students coming from Great Britain. In addition up to £50 per annum may be granted for travel in connection with research work.

Applications should be sent, not later than **February 1, 1974,** to the Secretary to Academic Council, The Queen's University, Belfast BT7 1NN, from whom application forms and further particulars may be obtained. The closing date for receipt of late applications is **March 1, 1974.** (449)

UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

RESEARCH FELLOW

Post-doctoral Research Fellow required as soon as possible until September 30, 1974, with the possibility of one or two years' extension to assist with studies on the effects of viruses on endocrine tissues. Medical or biological background is an advantage.

Salary range £1,929-£2,718 according to age and qualifications.

Application, with names of two referees, should be made to Secretary of Science, Science Office (E), University of Sussex, from whom further details can be obtained. (442)

UNIVERSITY OR WARWICK

POSTDOCTORAL FELLOWSHIPS IN STRUCTURAL AND ORGANIC CHEMISTRY

Applications are invited for two postdoctoral fellowships in the Department of Molecular Sciences, one in X-ray crystallography and the other in Organic Chemistry. Both fellowships are supported by the SRC. Post 'A' will involve the synthesis of inorganic compounds and determination of structure by single X-ray crystal methods using a Syntex 4-circle diffractometer. Ideally the person appointed will be experienced in synthesis and manipulation of air or heat-unstable compounds, preferably with some experience of X-ray methods. Post 'B' is directed towards methods of carrying out new selective organic transformations, and a background in physical organic or synthetic organic chemistry would be required. Work will involve either the preparation of novel micellating agents and study of their catalytic properties in solution or the control of thermal alicyclic reactions in fluoro or methoxyl-substituted molecules, depending on the background and aptitudes of the person appointed. Both appointments will be for one year. Salary scale £1,923 to £2,058 per annum with F.S.S.U.

Applications in writing (no forms) to the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No.: 25/4Q/74, as soon as possible indicating for which post they wish to be considered. Starting dates are flexible between March 1 and September 1, 1974. (357)

NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a POSTDOCTORAL RESEARCH FELLOWSHIP supported by the MRC for work on inositol lipid metabolism and diabetic neuropathy. Both animal work and clinical studies will be involved and technical assistance is available. Experience in neurochemistry, phospholipid metabolism or carbohydrate metabolism would be helpful. The appointment is for three years in the salary range £1,929 to £2,388 from a date to be agreed between April 1 and October 1, 1974.

Applications, with curriculum vitae and names of two referees to Professor J. N. Hawthorne, Department of Biochemistry, The Medical School, University of Nottingham, University Park, Nottingham NG7 2RD, from whom further details may be obtained. (362)

BEIT MEMORIAL FELLOWSHIPS FOR MEDICAL RESEARCH—1974

Notice is hereby given that an election of Junior Fellows to begin work on October 1, 1974, will take place in May 1974. Junior Fellows have annual values within the range of £1,750 to £2,220 per annum, plus yearly increments of £150 for 3 years. As a rule, superannuation benefits are provided, to which the Trust will make a contribution of 10 per cent. Candidates must have taken a degree in a faculty of a university approved by the Trustees in Her Majesty's Dominions, Protectorates and Mandated Territories, India, Pakistan, the Republic of Ireland and the United Kingdom, or a medical diploma registrable in the U.K. Elections to Junior Fellowships are rarely made above the age of 35 years. Applications from candidates must be received not later than March 22, 1974. Candidates must submit evidence that they can be given accommodation in the departments where they propose to work, which must be in Great Britain or Ireland.

Forms of application and all information may be obtained from Professor W. S. Spector, Pathology Department, St. Bartholomew's Hospital, London EC1A 7BE. For overseas candidates forms of application may be obtained from the Secretary, South African Medical Council, P.O. Box 205, Pretoria, South Africa; The Ministry of Health, The Government of India, New Delhi, India; the Secretary, Department of Education and Science, P.O. Box 826, Canberra City, A.C.T. 2601, Australia; The Department of Health, Wellington, New Zealand; and The Canadian Medical Association, 1867 Alta Vista Drive, Ottawa 8, Ontario, Canada. (439)

UNIVERSITY OF ADELAIDE

POSTDOCTORAL RESEARCH FELLOWSHIPS

The University will award a number of postdoctoral Research Fellowships in 1974 and 1975. These may be held in any department of the University; however, Fellowships are already held in the following departments: agricultural biochemistry and soil science, chemical engineering, computing science, education, genetics, geology, mathematical physics, organic chemistry, physical and inorganic chemistry, physics, plant pathology, pure mathematics, and zoology.

THE NORMAL AGE LIMIT will be 30 years, and preference will be given to an applicant who has qualified for the degree of Ph.D. or its equivalent within the last three years either elsewhere in Australia or in an overseas country.

TENURE will be for two years, with the possibility of extension for a third year.

SALARY SCALE: $A6,967-$A353 (4)-$A8,379.

Up to $A500 will be provided towards the cost of travel in coming to Adelaide. Up to the same amount may be provided if the Fellow should wish to return to his home state on the expiration of his Fellowship and has no appointment which provides for travel and removal expenses.

APPLICATIONS, in triplicate, must be made on the prescribed form. Forms and a leaflet outlining the conditions of appointment may be obtained from the Registrar, University of Adelaide, South Australia 5001, Australia. Applications are considered twice yearly and must be lodged with the Registrar by **28 February** or **1 July 1974.** (418)

QUEEN MARY COLLEGE

UNIVERSITY OF LONDON

Applications are invited for a POSTDOCTORAL FELLOWSHIP for X-ray structural studies in the pyrrole series in collaboration with Dr. R. Bonnett and Dr. M. B. Hursthouse. Appointment for one year in the first instance, but is extendable to two years. Salary within range £1,929-£2,223 per annum, plus £162 London Allowance.

Applications, in writing (giving qualifications, previous scientific experience, and names and addresses of two referees), to the Registrar (N), Queen Mary College, Mile End Road, London E1 4NS. (417)

SCOTTISH HORTICULTURAL RESEARCH INSTITUTE

INVERGOWRIE, DUNDEE DD2 5DA

ARC RESEARCH STUDENTSHIP

Applications are invited for an Agricultural Research Council studentship at the Institute. The award, tenable from October 1974, is intended to enable study for a higher degree and will involve research on the growth and development of the raspberry plant.

Candidates should have graduated with first or upper second class honours in a relevant subject. Consideration will also be given to students graduating in 1974.

Application forms and further particulars are available from the Secretary of the Institute. The closing date for applications is March 31, 1974. (399)

UNIVERSITY OF SALFORD

DEPARTMENT OF PURE AND APPLIED PHYSICS

RESEARCH FELLOWSHIP

Applications are invited for an S.R.C.-SPONSORED RESEARCH FELLOWSHIP tenable for three years in the Department of Pure and Applied Physics.

The research is concerned with the preparation of single crystals of rare earth intermetallic compounds and an investigation of their magnetic properties by neutron scattering techniques.

Salary scale: £3,378 rising to £3,708 a year. F.S.S.U.

Further details and application forms may be obtained from the Registrar, University of Salford, Salford M5 4WT, to whom completed applications should be returned by March 1, 1974, quoting reference P/139.

Informal applications can be made in the first instance to the Chairman of Department, Professor R. R. Birss. (385)

THE NUFFIELD FOUNDATION

TRAVELLING FELLOWSHIPS IN MARINE BIOLOGY (TROPICAL AND SUB-TROPICAL)

The Nuffield Foundation offers two Fellowships per annum to enable biologists in U.K. universities to travel overseas (normally for a period of between six and twelve months) to gain scientific experience of inshore marine environments in tropical and sub-tropical regions. Consideration can also be given to applications from physical scientists and from candidates who may be working in institutions other than universities. The primary aim of the scheme is to enliven and to broaden the scope of biology teaching at the tertiary level. The Fellowships are open to men and women holders of U.K. or other Commonwealth university degrees. The value of the awards will be determined on an *ad hoc* basis but will cover fares, subsistence allowances, and contributions to equipment and running expenses.

Applications, for awards to be taken up in 1975, should be submitted by March 31, 1974. Further particulars and forms of application are available from the Nuffield Foundation, Nuffield Lodge, Regent's Park, London NW1 4RS. (264)

GERHARD HERZBERG FELLOWSHIPS

UNIVERSITY OF SASKATCHEWAN, SASKATOON AND REGINA

In honour of Dr. Gerhard Herzberg, Nobel Laureate 1971 in Chemistry, and to commemorate his service to the University of Saskatchewan, the University is offering two Graduate Fellowships valued at $5,000 per annum renewable for a second year, one Fellowship tenable at the Regina Campus and one at the Saskatoon Campus. Applicants must have high academic qualifications and be enrolled, or be eligible to enrol, as a candidate for a graduate degree in the basic physical sciences. There is no citizenship requirement.

Inquiries and requests for application forms should be addressed to:

Dean, University College of Graduate Studies and Research, University of Saskatchewan, Regina, Canada S4S 0A2.

Deadline for completed applications is April 1, 1974, for Fellowship tenure to begin either May 1 or September 1, 1974. (436)

UNIVERSITY OF NOTTINGHAM

RESEARCH FELLOWSHIP IN FARM MANAGEMENT

Applications are invited for the above post in the Department of Agriculture and Horticulture. A working knowledge of modern management methods is essential and applicants will ideally have a good honours degree and some graduate research experience. It will be possible to register for a higher degree. Salary in the range £1,929 to £2,388.

Full curriculum vitae should be sent to the Secretary, School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD. (364)

UNIVERSITY OF BIRMINGHAM

FACULTY OF MEDICINE AND DENTISTRY

DEPARTMENT OF PHYSIOLOGY

Applications are invited for a POSTDOCTORAL RESEARCH FELLOWSHIP to collaborate in a study of the physiological and pharmacological regulation of renin release under the supervision of Dr B. Singer.

Starting salary up to £2,553 per annum plus F.S.S.U., depending on age and experience.

Applications, with curriculum vitae, naming two referees, should be sent to Professor S. M. Hilton, Head of the Department of Physiology, The Medical School, Birmingham B15 2TJ. (358)

NATIONAL INSTITUTE OF AGRICULTURAL BOTANY

TRAINEE OFFICER

Applications are invited from graduates with an honours degree in Agriculture, Agricultural Botany or Botany for an appointment as Trainee Officer in the Systematic Botany Branch of the Institute. Commencing salary £1,499 per annum. F.S.S.U.

Application form and further particulars from the Establishments Officer, N.I.A.B., Huntingdon Road, Cambridge CB3 0LE. Closing date February 14, 1974. (464)

NORTHERN REGIONAL HOSPITAL BOARD (SCOTLAND)

Applications are invited for the newly established post of BASIC GRADE BIOLOGIST (GRADUATE) in the Pathology Department of the Regional Hospital Board's Laboratories at Inverness. If possible prospective candidates should have special experience in cancer chemotherapy screening and/or tumour immunology. Salary in accordance with Whitley Council agreements.

Further particulars and forms of application are obtainable from the Senior Administrative Medical Officer, Northern Regional Hospital Board, Reay House, 17 Old Edinburgh Road, Inverness, with whom applications should be lodged by March 4, 1974. (467)

THE MACAULAY INSTITUTE FOR SOIL RESEARCH

DEPARTMENT OF SOIL FERTILITY

A PHYSICAL or INORGANIC CHEMIST is required to undertake studies on the nutrient status and relevant properties of soils, as part of a research programme on soil-plant relationships. The minimum qualification is a first or upper second class honours degree in Chemistry or equivalent, preferably with some postgraduate research experience.

The appointment will be in the Scientific Officer (£1,590 to £2,177) or Higher Scientific Officer (£2,076 to £2,667) grade, according to qualifications and experience, with superannuation under F.S.S.U., a non-pensionable allowance being added to salary to offset personal contributions.

Forms of application and further information may be obtained from The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ, to whom the completed forms should be returned before March 1, 1974. (Quote Ref. 74/4.) (469)

DEPARTMENT OF BIOLOGY, COLLEGE OF SCIENCE

UNIVERSITY OF MOSUL MOSUL, IRAQ

Applications are invited for teaching positions in chordates (vertebrate morphology), algae, archegonates and animal physiology for next academic year starting from September 1, 1974. Applicants should be holders of Doctorate degree and possess at least five years of university teaching and research experience in their respective fields. Salary will be fixed according to qualifications.

Detailed application, including a curriculum vitae and naming two referees, should be sent by airmail to Dr. M. H. Kasim, Head of the Department, at the earliest. (X426)

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF PHYSIOLOGY

RESEARCH ASSISTANT required for M.R.C. supported investigation of normal and abnormal cerebral vascular permeability in foetal and newborn animals. Full-time for minimum of 2 years. Postgraduate or postdoctoral in biological or medical sciences. Salary according to qualifications and experience.

Application and curriculum vitae to Dr N. R. Saunders (N), Department of Physiology, University College London, Gower Street, WC1E 6BT. (457)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

CANADA

DEPARTMENT OF BIOLOGY

Applications are invited for the position of BACTERIAL SYSTEMATIST. The successful candidate must have a good background in Bacterial Genetics and either Bacterial Physiology or Immunology. Appointment will be made at the Assistant Professor level. Applicants should have a strong interest in undergraduate teaching and be capable of initiating an independent research programme.

Apply to Professor J. Phipps, Head, Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada. (450)

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF PURE MATHEMATICS

Applications are invited for the post of LECTURER in the above Department, tenable from October 1, 1974. Salary in the range £1,929-£4,548 a year with F.S.S.U. provision.

Salary scales: Senior Lecturer, £4,368-£5,496; Lecturer, £1,929-£4,548.

Further particulars from the Registrar and Secretary, The University, Sheffield S10 2TN, to whom applications (five copies) should be sent by February 16, 1974. Please quote Ref. R.24/G. (420)

UNIVERSITY OF WARWICK

PROFESSORSHIP OF ENVIRONMENTAL SCIENCES

Applications are invited for a new post of Professorship of Environmental Sciences tenable from October 1, 1974. Salary in the Professorial range (minimum £5,625 p.a.). Superannuation and removal allowance payable.

Further particulars may be obtained from the Academic Registrar, University of Warwick, Coventry CV4 7AL, to whom applications (3 copies) naming three referees, should be sent by February 28, 1974. Please quote Ref. No. 26/Q/74. (443)

QUEEN ELIZABETH COLLEGE

(UNIVERSITY OF LONDON)
Campden Hill Road, W8 7AH

RESEARCH TECHNICIAN

required to operate and maintain Chemistry Department AEI MS 30 Mass Spectrometer. The instrument provides the ULIRS gas chromatography/mass spectrometry service for London University. An AEI DS 50 Data Acquisition System will be added in April.

Applications, with the names of two referees and a note of qualifications and any relevant experience, to Dr. A. J. MacLeod at the above address. (410)

INSTITUTE OF OPHTHALMOLOGY

(UNIVERSITY OF LONDON)
JUDD STREET, LONDON WC1H 9QS

A vacancy exists for a young IMMUNOLOGIST, preferably post-doctoral, with experience in cell-mediated immunity, to join a project of research on the role of cell-mediated immune mechanisms in human chlamydial disease. This work is associated with clinical and laboratory projects on ocular and genital chlamydial disease in London and hyperendemic trachoma in Iran. The appointment will be for three years in the first instance. Commencing salary £2,058 plus £162 London allowance.

Applications with full details of qualifications and experience, together with names and addresses of two referees to the Secretary at the above address by February 8, 1974. (400)

UNIVERSITY OF DURHAM

DEPARTMENT OF MATHEMATICS

Applications are invited for the post of TEMPORARY LECTURER IN APPLIED MATHEMATICS for one year from October 1, 1974, during the absence of Dr P. Goddard on a Fellowship at Princeton.

The salary will be on the scale £1,929 to £4,548 per annum with F.S.S.U. benefits.

Applications (3 copies) including the names and addresses of three referees should be sent by February 11, 1974, to the Registrar and Secretary, Science Laboratories, South Road, Durham, from whom further particulars may be obtained. (367)

JUNIOR TECHNICAL OFFICER REQUIRED by Institute of Cancer Research in the Immunobiology Department of the Chester Beatty Research Institute, Fulham Road, London SW3. Two 'A' level science subjects are the minimum qualification, and experience in tissue culture methods would be an advantage. The work will be on a project designed further to explore the nature and functions of the lymphoid system in mice. Salary on M.R.C. scales.—Apply in duplicate, giving names of two referees, to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting Ref. No. 301/B/499. (405)

RESEARCH TECHNICIAN required to assist with the radioimmunoassay of Angiotensin I. This will be as part of a small research team investigating the physiological action of renin in children. The post is tenable for two years and would be suitable for a qualified technician who wishes to obtain radioimmunoassay experience. Newly qualified graduates would also be considered but the post is not suitable for a higher degree. Salary to £1,509 plus £126 L.W.—Applications to Dr M. Dillon, Department of Chemical Pathology, The Institute of Child Health, 30 Guilford Street, WC1N 1EH. Enquiries 242 9789 Ext. 121. (462)

UNIVERSITY OF EAST ANGLIA

Applications are invited for two posts for research on growth of the alga Prymnesium parvum in the Norfolk Broads. One post is for a POST-DOCTORAL SENIOR RESEARCH ASSOCIATE who will study the physiological ecology of the organism, and the other for a JUNIOR RESEARCH ASSOCIATE to investigate its seasonal cycle and the underlying causes of it. Starting salaries will be £1,929 plus F.S.S.U. for the senior post and £920 for the junior post. The latter will be permitted to register for a higher degree. Both posts will be for one year initially, but renewable for a further two years.

Further information is available from Dr. Brian Moss, School of Environmental Sciences, University of East Anglia, Norwich NOR 88C, to whom applications, quoting two referees, should be sent by February 11, 1974. (404)

UNIVERSITY OF ABERDEEN

RESEARCH ASSISTANT: MEDICAL PHYSICS

Applications are invited for above post for work with the Radiation Protection Section of the Department of Medical Physics but primarily in the Aberdeen Royal Infirmary. Duties include supervising the safe distribution and administration of all radioisotopes prepared in the Medical Physics Department for hospital use. Training will be given but a mature person with adequate scientific background and experience of work in a responsible position is essential.

Salary on scale: Grade II, £1,662 to £2,304 with placing according to qualifications and experience.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by February 19, 1974. (448)

MEDICAL RESEARCH COUNCIL

RADIOBIOLOGY UNIT
HARWELL, BERKSHIRE

TECHNICAL ASSISTANT (BIOLOGIST) wanted. Excellent opportunity for young graduate to join a research group investigating genetic effects of radiation and other agents in small animals. Varied work involving animal genetics, cytogenetics, tissue culture, etc. Salary according to age and qualifications. Fringe benefits include modern hostel accommodation for single men and women at a reasonable cost, excellent social facilities and proximity to Oxford.

Applications, in writing, to: The Administrator, Medical Research Council, Radiobiology Unit, Harwell, Berkshire, OX11 0RD. Reference MFL/34. (468)

FELLOWSHIPS AND STUDENTSHIPS

UNIVERSITY OF DUNDEE

DEPARTMENT OF CHEMISTRY

Applications are invited for an **S.R.C. sponsored Postdoctoral Fellowship** for work with Dr. A. Miller on a study of reaction mechanisms in organophosphorus chemistry.

The appointment is available from April 1, 1974, and will be for either one or two years. Salary £2,058 by £165. F.S.S.U.

Applications, in writing, quoting Reference Est/19/74J, and containing the names and addresses of two referees, should be sent by March 1, 1974, to the Secretary, The University, Dundee DD1 4HN. (414)

UNIVERSITY OF ESSEX

DEPARTMENT OF CHEMISTRY

Applications are invited for RESEARCH FELLOWSHIPS in each of the following fields:

1. Crossed molecular beam studies of elementary reactions (experience of scattering techniques would be of considerable advantage).

2. Chemi-ionization studies by electron spin resonance (experience in spectroscopy would be of value).

3. Shock tube studies of combustion-generated pollution.

4. Shock tube studies of initiation in fuel-air mixtures.

Candidates are expected to hold an appropriate qualification such as an Honours degree in chemistry or physics and have three or more years' research experience, although candidates with less experience may be considered.

Further details are available from Professor J. N. Bradley, Department of Chemistry, University of Essex, Wivenhoe Park, Colchester CO4 3SQ. Telephone 0206 44144, Ext. 2030. (408)

UNIVERSITY OF DUNDEE

Technical or Senior Technical Officer in the Physics Department

to join research group working on electronic and optical properties of amorphous solids under the direction of Professor W. E. Spear. Applicants should have postgraduate experience in this field. The appointment is for two years from March 1, 1974, and is financed from an SRC grant. Salary in the range £1,929-£2,883.

Applications, quoting reference Est/107/74J, and containing names and addresses of two referees, should be submitted as soon as possible to the Secretary, University of Dundee, Dundee DD1 5HN, from whom further information can be obtained. (446)

PURDUE UNIVERSITY

DEPARTMENT OF GEOSCIENCES

is seeking applicants for an expected faculty opening for the 1974-75 academic year. The position will be filled in the area of **atmospheric chemistry** at the rank of Assistant Professor or Associate Professor. The candidate is required to possess a Ph.D. in addition to formal academic background or experience in both atmospheric science and chemistry. This position is intended to provide for the initiation of an atmospheric chemistry programme at both the undergraduate and graduate level. Candidates for this faculty opening will therefore be judged both with respect to their teaching and personal research ability, and with respect to their ability to guide and support graduate student research. Because of the expected involvement with this new research area, only highly qualified individuals will be considdered.

Letter applications should be sent before February 28, 1974, to: Dr. Gunnar Kullerud, Head, Department of Geosciences, Purdue University, West Lafayette, Indiana 47907. They should include a comprehensive resume, a listing of all publications, and the names of three references. Non-citizens should describe their immigration status. Purdue University is an equal opportunity—affirmative action employer. (403)

UNIVERSITY OF PAPUA NEW GUINEA

Applications are invited for the post of PROFESSOR OF PHYSICS. Appointee should be interested in Science Education, its particular problems in Papua New Guinea, and the extensive role played by Physics to Agriculture, Medicine, General Science and Education students at elementary levels. The present interdisciplinary research by the Department is in the general area of environmental physics. Specific research interests are in Geophysics, Meteorology, Micro-meteorology, Soil Physics and Instrumentation, but the interests of the Professor, need not necessarily be confined to these specialities.. He might be expected to have a broad interest in environmental research and extensive experience in at least one aspect of it. The position is available from October 1974 or as soon as possible thereafter.

Salary: $A16,306 per annum plus $A360 per annum dependants' allowance. (This includes an overseas allowance.) (£1 sterling=$A1.50.) Conditions include provision of housing, annual leave fares, F.S.S.U. superannuation. Applications (2 copies) should include particulars of age, nationality, marital status, qualifications and experience, names and addresses of three referees, a recent small photograph and an indication of estimated starting date.

Further details and the conditions of appointment are available from K. R. Long, Secretary, P.O. Box 4820, University, Papua New Guinea. Closing date March 31, 1974. (458)

UNIVERSITY OF ZURICH

CYTOLOGY LABORATORY, BOTANY DEPARTMENT

A POSTDOCTORAL RESEARCH POSITION for work on the biochemistry of differentiation in cellular slime moulds (Dictyostelium Polysphondylium) is available immediately. The appointment is for 2 to 3 years and the salary ca. SFr. 30,000 per annum.

Applications, with curriculum vitae and the names of two referees, should be sent to Prof. H. R. Hohl, Cytologisches Laboratorium, Universität Zürich, 8050 Zürich, Birchstrasse 95, Switzerland. (461)

ROYAL POSTGRADUATE MEDICAL SCHOOL

TECHNICIAN/SENIOR TECHNICIAN required for work on the virology of patients with infections of the heart. Experience in virology (Serology and cell culture in particular) is required. Appointee will also be expected to participate in the work of the routine clinical virology section. Qualifications: HNC or equivalent. Salary according to qualifications and experience.

Applications to the Secretary, R.P.M.S., Hammersmith Hospital, DuCane Road, London W12 0HS, quoting Ref. 10/246/N. (454)

ROYAL POSTGRADUATE MEDICAL SCHOOL

(HAMMERSMITH HOSPITAL) DEPARTMENT OF HAEMATOLOGY

Applications are invited for the following posts:

1. TECHNICIAN in blood transfusion department. A wide selection of routine tests and specialised work is carried on, providing excellent opportunities to study for the advanced blood transfusion exam of the I.M.L.T. Ref. 7/114/N.
2. TECHNICIAN in haematology department. The range of work gives opportunity to gain wide experience in all the aspects of haematology required for the advanced exam of the I.M.L.T. Ref. 7/111/N.
3. TECHNICIAN/GRADUATE TECHNICAL OFFICER required to work on development and routine aspects of quality control in haematology. Ref. 7/269/N.
4. TECHNICIAN with training in electron microscopy required for haematology quality control project. Ref. 7/228/N.

Classes for advanced exams are held at the School. The salary and grading for these appointments will be according to qualifications and experience.

Applications to the Secretary, R.P.M.S., Hammersmith Hospital, DuCane Road, London W12 0HS, quoting the appropriate Ref. (455)

ST. THOMAS' HOSPITAL

LONDON SE1 7EH

PRINCIPAL GRADE HOSPITAL BIOCHEMIST

£3,708–£4,836+£126 L.W.

For the Section of Clinical Chemistry, which is part of the Medical School's Department of Chemical Pathology, and provides a routine service for the hospital's patients. A successful applicant would be the most senior graduate in clinical chemistry and would be expected to take a major part in developing and improving the scientific aspect of the department, as well as sharing administrative functions. Whitley Council terms and conditions of service. Visiting may be arranged with Dr. McSwiney (01-928 9292, Ext. 2267).

Applications, quoting Ref. PBC/CC, should be sent to the Personnel Officer, 79 York Road, London SE1 7NY, before February 28, 1974. (411)

ROTHAMSTED EXPERIMENTAL STATION

HARPENDEN, HERTS AL5 2JQ

SCIENTIFIC OFFICER to work in the Entomology Department on aspects of insect behaviour leading to the improvement of current methods of pest control. The work will consist initially of establishing and maintaining insect cultures, with opportunities to participate in other laboratory and field research. Minimum qualifications: degree in entomology, zoology or invertebrate physiology, H.N.C., or the equivalent in a biological subject. Starting salary £1,318-£1,590, according to qualifications and experience, on a scale to £2,177. Superannuation, with non-pensionable allowance to offset personal contributions.

Applications, naming two referees and quoting Reference 2192, to the Secretary by February 11, 1974. (412)

HAMPSHIRE AND ISLE OF WIGHT NATURALISTS' TRUST LTD.

CONSERVATION OFFICER

Applications are invited for the post of Conservation Officer. Candidates should have practical experience of conservation management, a sound knowledge of ecology, and organising ability. Salary in the range £1,500 to £2,500, according to experience and qualifications.

Further particulars may be obtained from the Secretary, King John's Lodge, Romsey, SO5 8BT, to whom applications should be sent, together with curriculum vitae and the names and addresses of three referees, to arrive by March 1, 1974. (465)

JOHN INNES INSTITUTE

GENETICIST

The Institute will shortly appoint a Geneticist to undertake a programme of research on somatic cell genetics in plant tissue culture. The successful applicant will work in the Department of Applied Genetics which already has a number of projects on anther and tissue culture and will have close contact with a number of projects on plant protoplasts being undertaken in other departments.

Candidates should have a good honours degree and experience in microbial or higher plant genetics.

The appointment, which will be for two years, will be to the Higher Scientific Officer (£2,076 per annum to £2,667 per annum) or Senior Scientific Officer (£2,615 per annum to £3,640 per annum) grade according to experience. Membership of the F.S.S.U. is obligatory.

Applications, including full curriculum vitae, publication record and the names of two referees, should be forwarded to the Secretary, John Innes Institute, Norwich, NOR 7OF, by February 4, 1974. (463)

UNIVERSITY OF LANCASTER

Applications are invited from suitably qualified persons for the post of LECTURER in ATMOSPHERIC CHEMISTRY in the Department of Environmental Sciences.

The successful candidate will be expected to develop research programmes in atmospheric pollution and related topics. Experience in meteorology would be an advantage but is not essential.

Further particulars may be obtained (quoting reference L. 794/N) from the Establishment Officer, University House, Bailrigg, Lancaster LA1 4YW, to whom applications (five copies), naming three referees, should be sent not later than February 28, 1974. (371)

UNIVERSITY OF SHEFFIELD

CHAIR OF APPLIED MATHEMATICS

Applications are invited for the Chair of Applied Mathematics vacant on the appointment of Professor P. C. Kendall to the Headship of the Department of Mathematics at the University of Keele. Salary in the range approved for professorial appointments with F.S.S.U. provision.

Salary scales: Senior Lecturer, £4,368-£5,496; Lecturer, £1,929-£4,548.

Further particulars may be obtained from the Registrar and Secretary, The University, Sheffield S10 2TN, to whom applications (one copy only) should be sent by February 25, 1974. Quote Ref. 22/G. (368)

UNIVERSITY OF ABERDEEN

RADIOISOTOPE PHYSICISTS

Applications are invited for two posts in the Department of Medical Physics, preferably with hospital experience, to join team providing hospital services in nuclear medicine, postgraduate teaching and research/development in radionuclide imaging, both clinical and instrumentation.

Salary on scale: £1,929 to £4,548 for one post at Lecturer level, £1,662 to £2,304 or £2,310 to £3,093 at more junior level, all with placing according to qualifications and experience.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by February 19, 1974. (447)

CHARING CROSS HOSPITAL MEDICAL SCHOOL

GRADUATE/TECHNICIAN required for a varied research programme in the Department of Obstetrics and Gynaecology, involving Haematology, and some Biochemistry and Histochemistry. Previous experience not essential. Salary £1,500 per annum, post tenable for one year in the first instance with probable extension for a suitable individual. Please apply with curriculum vitae to: The Secretary, Charing Cross Hospital Medical School, Brandenburgh House, Fulham Palace Road, W6 9HH. (392)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)

DENMARK HILL, LONDON SE5 8RX

SENIOR RESEARCH BIOCHEMIST required to join multidisciplinary group in a M.R.C. supported programme into the development of the artificial liver with particular reference to brain metabolism. Ph.D. preferred. Salary within the range £2,388 to £3,048 plus £162 London allowance.

Further particulars from Dr. R. Williams, Liver Unit. Applications to the Secretary of the Medical School not later than February 8, 1974. (396)

ST. BARTHOLOMEW'S HOSPITAL

LONDON E.C.1

GASTROENTEROLOGY ASSISTANT required to help and supervise patients for investigations. Medical qualifications not necessary.

Salary scale £708 to £1,335 per annum plus £126 London Weighting.

Please apply in writing to the Personnel Officer, quoting ASC/3872/N. (470)

UNIVERSITY OF CALGARY, GRADUATE Teaching and Research Assistantships are available for students proceeding to the Ph.D. or M.Sc. degrees in all branches of Chemistry and Biochemistry. The Assistantships are valued from $4,100 to $5,000 per annum and carry remission of programme fees and travel allowance.

For further information write to: Department of Chemistry, Calgary, Alberta T2N 1N4, Canada. Please indicate field of interest. (X35)

Kings
COLLEGE HOSPITAL GROUP

Cytogenetics, Department of Haematology

GRADUATE SCIENTIST

to work in the Genetic Laboratory of this busy teaching hospital. The post offers experience in the application of a wide variety of modern techniques to the diagnosis and management of inherited and malignant disorders. Experience in tissue culture or cytogenetics an advantage.

Application forms available from: Personnel Officer, King's College Hospital, Denmark Hill, London SE5 9RS. Tel.: 01-274 6222, Ext. 2724/8. (365)

CHAIR OF PATHOLOGY

Medical college and large teaching hospital in major medical centre is considering applications for the Chair of Pathology. Rush-Presbyterian-St. Luke's Medical Center is an equal opportunity/affirmative action employer.

For further information please contact Dr Friedrich Deinhardt, Chairman of the Search Committee, Department of Microbiology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois 60612. (466)

AUSTRALIAN NATIONAL UNIVERSITY

CHAIR OF BIOCHEMISTRY

Applications are invited for appointment to the Chair of Biochemistry in the Faculty of Science. The Chair became vacant in November 1973 when Professor L. M. Birt resigned to take up the appointment of Vice-Chancellor Designate of Wollongong University College, New South Wales. It is hoped that the appointee will be able to take up duty in 1974.

The Department of Biochemistry offers courses leading to the pass and honours degree of Bachelor of Science and supervises students for the degrees of Master of Science and Doctor of Philosophy. Undergraduate teaching in biochemistry and, to a limited extent in microbiology, begins in the second year of the Bachelor of Science course. Seven semester courses at the second- and third-year level are offered. There are seven members of the academic staff (Lecturers and above).

The salary for the post is $A19,102 per annum. The University provides reasonable travel and removal expenses and assistance with housing for an appointee from outside Canberra. Superannuation is on the F.S.S.U. pattern with supplementary benefits. Financial assistance towards study leave is also available.

The University reserves the right not to make an appointment or to make an appointment by invitation.

Further information and the forms which should accompany any application may be obtained from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close on **March 25, 1974.** (451)

UNIVERSITY OF CAPE TOWN

PROVINCIAL ADMINISTRATION OF THE CAPE OF GOOD HOPE

LECTURER IN PHYSIOLOGY

Applications are invited for the above post, vacant as from July 1, 1974, in the Department of Physiology and Medical Biochemistry. Appointment, according to qualifications and experience, will be made on the salary scale R5,700 by R300 to R8,100 per annum, plus a pensionable allowance of 15 per cent of basic salary.

Training and experience in neurophysiology will be an advantage. The duties include systematic teaching of medical and science students and medical auxiliaries and supervision of laboratory classes. The timetable allows ample time for research.

Applicants should submit a curriculum vitae, stating present salary, research interests and publications, when available if appointed, and the names and addresses of three suitable referees.

Memoranda concerning the position and further information should be obtained from the Registrar, University Private Bag, Rondebosch, C.P., by whom applications must be received not later than March 15, 1974.

Appointment will be subject to a satisfactory medical examination. The University reserves the right to appoint a person other than one of the applicants or to make no appointment.

(394)

WESTERN REGIONAL HOSPITAL BOARD

DEPARTMENT OF CLINICAL PHYSICS AND BIO-ENGINEERING

ELECTRONIC ENGINEERS/ PHYSICISTS, BASIC GRADE

Applications are invited from Electronic Engineers or Physicists with an interest in electronic technology for two posts in this large, multi-disciplinary department which serves the hospitals of the Western region and will shortly serve the new health boards to be established in the west of Scotland. The duties combine participation in regular service responsibilities with opportunities to participate in development and research in many aspects of health care. Although it is expected that these entrants will make their main contribution in areas where electronics and allied technologies are important, new entrants will be encouraged in their early years to gain all round experience of bioengineering and clinical physics as a basis for a career.

Applicants should have a first or second class honours degree; and preferably some practical professional experience.

The starting salary depends on experience, e.g., £1,680 on graduation or £1,896 with three years of postgraduate study or experience. Subsequently it rises in this grade by annual increments to £2,517. There are good prospects of promotion to high grades after four years of postgraduate experience. Studies for higher qualifications are often possible.

Application forms and further information may be obtained from the Regional Physicist, Western Regional Hospital Board, Department of Clinical Physics and Bio-Engineering, 11 West Graham Street, Glasgow G4 9LF. (444)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF ORGANIC CHEMISTRY

Applications are invited for a post of POST-DOCTORAL RESEARCH ASSISTANT for work on a project, supported by the Science Research Council, concerned with chemically induced dynamic nuclear spin polarization (CIDNP) during reactions at chiral centres. Applicants should preferably have experience in NMR spectroscopy, radical chemistry or the synthesis and resolution of optically active compounds.

The appointment would be for one year in the first instance and would start as soon as possible, but not later than October 1, 1974. The initial salary will be £1,929 per annum plus FSSU.

Applications, stating age, qualifications and experience, together with the names of two referees, should be received not later than March 31, 1974, by the Registrar,' The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref. RV/N/80668.

(445)

THE LONDON HOSPITAL MEDICAL COLLEGE

(UNIVERSITY OF LONDON)

RESEARCH ASSISTANT— GASTROENTEROLOGY

Applications are invited for the appointment of Research Assistant within the Medical Unit. Duties will involve the study of pancreatic function and the post would be suitable for a graduate holding a good honours degree with major subject in either Physiology or Biochemistry. Opportunities to study for higher qualification. Salary within the range £1,563-£1,722 plus £126 London Allowance per annum.

Applications, giving full details of education and qualifications, together with name of referee, to the Secretary, The London Hospital Medical College, Turner Street, London E1 2AD, quoting Ref: MUG/2/74. (453)

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOGY

TECHNICIAN, preferably qualified in haematology or related subjects, required for research in cellular aspects of immune reactions to participate in research in Immunology Division, Addenbrooke's Hospital, Hills Road, Cambridge. Salary in range £1,400-£1,900 according to age, experience and qualifications.

Applications in writing to the Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. (422)

University of Leeds

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a post of LECTURER. Salary scale £1,929 to £4,548.

Forms of application and further particulars from the Registrar, The University, Leeds LS2 9JT (please quote 44/12/D). Closing date March 22, 1974. (372)

GUY'S HOSPITAL MEDICAL SCHOOL

Research Assistant

with Honours degree in biochemistry, required to assist in the Paediatric Research Unit with research on treatment of the mucopolysaccharidoses. One or two years' postgraduate experience in enzyme purification, glycosaminoglycan chemistry, and cell culture techniques an advantage.

Applications should be sent to the Administrative Secretary, Paediatric Research Unit, Guy's Hospital Medical School, London Bridge, SE1 9RT. (421)

STANDARDS LABORATORY FOR SEROLOGICAL REAGENTS

TECHNICIAN A.I.M.L.T.

(Bacteriology or Virology) or H.N.C. in Medical Laboratory Sciences (Microbiology) required for work with viral diagnostic reagents. Graduates may apply for this position.

Written applications with full details of age, experience and qualifications to the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. (460)

THAMES POLYTECHNIC

SCHOOL OF ARCHITECTURE

LECTURER II IN ENVIRONMENTAL SCIENCE

The School of Architecture needs an environmental scientist, environmental engineer, or other suitably qualified graduate, to teach the application of environmental science to building. Applications from recently qualified graduates who have an interest in this field will be welcomed.

The successful candidate will be a member of the teaching Division of Construction Technology, which is part of the Faculty of Architecture and Surveying, located at Hammersmith, London W.6, and will also be associated with teachers of Physics and Materials Science at the Woolwich site of the Polytechnic. Personal interests and research in solar radiation or acoustics will be encouraged.

The successful applicant will teach students on full-time diploma courses in Architecture, C.N.A.A. degree courses in Surveying, and on part-time courses and will be able to contribute to planning a degree course in Architecture shortly to be submitted to the C.N.A.A.

Salary scale: £2,515 to £3,243 plus £118 London allowance.

Further particulars and form of application may be obtained from the Secretary, Thames Polytechnic, Wellington Street, London SE18 6PF, to whom completed applications should be returned by February 12, 1974. (383)

UNIVERSITY OF OREGON

DEPARTMENTS OF PHYSICS AND GEOLOGY

The University of Oregon invites applications for an anticipated joint appointment in the Departments of Physics and Geology at the Assistant Professor level. Teaching and research interests for this position should be within broad scope of **general geophysics and planetology,** but potential for excellence and ability to communicate with both physicists and geologists are considered more important than area of specialization. The person appointed may also affiliate with Department of Geology's Center for Volcanology and/or the Interdepartmental Institute for Theoretical Science. The University is an equal opportunity employer.

Applications, including resume and names of three or more referees, should be sent to Professor W. T. Holser, Head, Department of Geology, University of Oregon, Eugene, Oregon 97403, U.S.A.

(389)

UNIVERSITY OF SUSSEX

School of Biological Sciences

RESEARCH ASSISTANT IN ANIMAL BEHAVIOUR

Applications are invited for the post of Research Assistant to help with a project on the temporal pattern and causation of behaviour in passerine birds. Candidates should preferably have a degree in Biology or Psychology, but those with a mathematical background and an interest in animal behaviour will also be considered.

The post is tenable for three years from March 1, 1974, with salary in the range £1,002×60-£1,362.

Further details and application forms may be obtained from the Secretary of Science, Science Office (E), University of Sussex, Falmer, Brighton BN1 9RH. (441)

THE QUEEN'S UNIVERSITY OF BELFAST

CHAIR OF PSYCHOLOGY

Applications are invited for the second Chair of Psychology created from October 1, 1974. The other Chair is held by the Head of the Department, Professor M. P. Haggard. The salary is £6,462 per annum with contributory pension rights under the F.S.S.U.

Applications should be received by April 1, 1974. Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland. (Please quote Ref. 74/N.)

(373)

THAMES POLYTECHNIC

SCHOOL OF BIOLOGICAL SCIENCES

DIVISION OF ENVIRONMENTAL BIOLOGY

Applications are invited for a

RESEARCH ASSISTANTSHIP

to undertake studies on the microbial dissimilation of the halogenated phenoxyacetate herbicides.

Applicants should hold a good honours degree in Microbial Biochemistry, Biochemistry or Chemistry. Industrial experience and some training in Microbiology are preferable though not essential.

The successful candidate will receive a starting salary of £1,427 per annum and will be expected to register for a higher degree.

Further details and application form may be obtained from the Secretary, Thames Polytechnic, Wellington Street, London SE18 6PF, to whom completed applications should be returned by February 12, 1974. (384)

UNIVERSITY OF ZAMBIA

Applications are invited for (a) SENIOR LECTURESHIP or (b) LECTURESHIP IN ANIMAL SCIENCES in the School of Agricultural Sciences. Applicants should have a good first degree, preferably in agricultural science, and postgraduate training to Ph.D. level in animal breeding and/or livestock management and breeding. Preference will be given to those with teaching experience in cattle and/or pig improvement and production. Appointee will be expected to teach undergraduate courses in the above-mentioned fields to students studying general agricultural science; to supervise and instruct these students during practical training; to develop postgraduate training in animal science; to initiate research relevant to Zambia's developing livestock industries; to assist generally in the running of the School of Agricultural Sciences. He will join an animal science team consisting of an animal physiologist, two animal nutritionists, and a veterinarian. The University is currently developing farm facilities, and the School is expected to move into a new laboratory/teaching complex in 1976. Salary scales: (a) K5,600-K6,600 per annum, (b) K4,000-K5,400 per annum. (£1 sterling=K1.43.) The British Government may supplement salary in range £200-£500 per annum (sterling) for married appointee or £100-£200 per annum (sterling) for single appointee (normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages, various allowances; superannuation and medical aid schemes; regular overseas leave.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be forwarded by air mail not later than February 25, 1974, to the Registrar, University of Zambia, P.O. Box 2379, Lusaka, Zambia. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London WIP 0DT. Further particulars may be obtained from either address. (419)

THE ANIMAL VIRUS RESEARCH INSTITUTE

PIRBRIGHT, WOKING, SURREY

VACCINE RESEARCH DEPARTMENT

Applications are invited for a post in the above Department. The successful candidate will work in a small team on the selection and development of new foot-and-mouth disease vaccine strains. The work will include assessment of new strains on the laboratory scale for growth properties and immunogenicity. Experience in virus work is essential, and interests in viral immunology and strain selection procedures would be an advantage. Applicants should have a good Honours degree in microbiology or allied subject and some years' experience as a postgraduate research worker. Appointment in S.S.O. grade (£2,615-£3,640), depending on age and qualifications. Superannuation under F.S.S.U., with allowance to offset cost of contributions. House available for suitable married applicant.

Further particulars and application forms from Secretary. (432)

UNIVERSITY OF NIGERIA, NSUKKA

Vacancies

FACULTY OF PHYSICAL SCIENCES

(Advert. Ref. No: UNP/SSA. I/FPS/STATS/73)

DEPARTMENT OF STATISTICS

(a) Professor **(b) Reader** **(c) Senior Lecturers**	interests in at least one of the following areas: Statistical Theory, Statistical Methods, Probability Theory, Applied Probability
(d) Lecturers/Asst. Lecturers, Nsukka/ Calabar Campuses	interest in statistics or probability

QUALIFICATIONS AND DUTIES:

Candidates for the Post of Professor, Reader, Senior Lecturer or Lecturer should possess advanced (preferably doctorate) degrees in relevant fields with University Teaching and research experience and evidence of productive research.

Successful candidates will teach courses to undergraduates and postgraduates as well as conduct research.

Candidates for Assistant Lectureship should possess a good first degree plus an advanced degree with some teaching/research experience in relevant fields. Successful candidates will teach undergraduate courses and participate actively in conducting research.

SALARY SCALES:

Professor	SG. 2—N6,600 (consolidated)
Reader	SG. 4—N6,100 (consolidated)
Senior Lecturer	SG. 5—N5,030 × 150–N5,480, N5,750
Lecturer	SG. 7—N2,760 × 150–N3,660 (bar): N3,810 × 150–N4,260, N4,530 × 150–N4,830.
Assistant Lecturer	SG. 12—N2,140 × 100–N2,460 × 100–N2,580 p.a. (OR N2,460 × 100–N2,660 for appointees over 28 with higher degrees).

CONDITIONS OF SERVICE:

Fare paid for appointee, wife and up to five children under eleven years of age on appointment; leave after 21 months' tour and termination. Children and car allowances, superannuation scheme, free medical services, part-furnished accommodation at standard rent rates.

METHOD OF APPLICATION

TEN copies of typewritten application giving details of educational background, institutions attended and dates, qualifications, teaching/research experience with institutions and dates, nationality, marital status, age, list of publications (if any), and names and addresses of THREE referees competent to attest to candidates' academic/professional abilities to: THE PERSONNEL OFFICER, University of Nigeria, Nsukka, quoting the appropriate advertisement reference.

(380)

SPORTS TURF RESEARCH INSTITUTE, BINGLEY, YORKSHIRE

AMENITY GRASS RESEARCH

Department of the Environment (Natural Environment Research Council Contract)

The Institute will shortly undertake (on contract with the Natural Environment Research Council on behalf of the Department of the Environment) two projects concerned with amenity use of grass (sport, lawns, landscaping and environmental management). Applications are invited for the following four posts based at Bingley:-

A **Project General Assistant** (Post I) will be for a two-year period, to help the senior staff of the Institute concerned with the first major review of amenity grass areas. The General Assistant will work closely with the Institute's research team. A degree in botany or related subject is desirable, with some aptitude for paperwork, and an adaptable outlook. Salary £1,402 to £2,177, possibly starting above the minimum.

Two **Turfgrass Trials Officers** (Posts 2 and 3) will be appointed to take charge of a network of extension trials for assessing cultivars and turfgrass mixtures. It is planned to start these trials as soon as possible at several centres throughout the U.K. Candidates should preferably have at least a 2nd Class Honours degree in an appropriate subject in addition to relevant postgraduate experience. They should have some knowledge of grasses, experience of working on their own and supervising others, and be prepared for frequent travelling.

Salaries £2,076 to £2,667 or £2,165 to £3,640, depending on qualifications.

A **Headquarters Trials Officer** (Post 4) will help in developing the existing cultivar and mixture trials at Bingley to reinforce and complement the national testing network; he will be responsible for assessing the trials and undertaking limited special investigations in connection with them. Candidates should preferably have a good degree in a relevant subject; research experience, preferably with grasses, would be an advantage. Salary £1,402 to £2,177 or £2,076 to £2,667.

Further information and application forms from The Secretary, Sports Turf Research Institute, Bingley, Yorkshire BD16 IAU.

(381)

THE MALAYSIAN RUBBER PRODUCERS' RESEARCH ASSOCIATION

requires an experienced

ELECTRON MICROSCOPE TECHNICIAN

to take charge of the operation and maintenance of its Siemens Elmiskop Mk 1 and ancillary equipment. The position is suitable for candidates aged between 25 and 35 with experience in biological and physical work and with a sound knowledge of replica embedding and sectioning techniques. The starting salary may be between £1,400 and £2,600 per annum, depending on age, experience and qualifications. There is a pension scheme.

The Association will be moving in mid-1974 to an attractive new location on a 30-acre site near Hertford. Transport from Welwyn Garden City and locality may be provided.

Please write giving details of qualifications and experience to:

The Director,
The Malaysian Rubber Producers' Research Association,
56 Tewin Road,
Welwyn Garden City, Herts.

(429)

Research in Physical Sciences Culham Laboratory

Expansion of contract research work in the Applied Physics and Technology Division at Culham Laboratory creates the need for additional scientific staff. Expansion is taking place in the work on industrial electrostatics and in the investigation and development of field emission thrustors for satellite position and altitude control. The work on electrostatics is at present concerned with assessment of practical hazards—in particular relating to tanker explosion—and with electrostatic precipitation. Work on field emission thrustors is concerned with investigation of field ion emission characteristics of liquid caesium films on needle arrays. The above areas require additional staff with the ability to carry out independent experimental and theoretical research, the ability to take initiative in discussing project work with customers and the interest and enthusiasm to initiate new areas of activity.

Candidates with a good honours degree in physical science are invited to write to the Personnel Officer, UKAEA Research Group, Culham Laboratory, Abingdon, Berkshire, OX14 3DB (Ref. A469) for application forms.

Salaries for 3-year appointments will normally lie in the range £1,415 to £3,895 according to qualifications, experience and age.

UKAEA
CULHAM LABORATORY

Culham
Laboratory

(377)

Swiss Federal Institute of Technology, Zurich

At our Department of Geography we are looking for an

Associate Professor (ausserordentlicher Professor) of Quantitative Geography

Applicants for this new post should have teaching and research experience in the field of mathematical treatment of geographical problems, particularly in the handling of data and models by computer, in both physical and human geography.

Applications, including curriculum vitae, evidence of research ability and teaching experience, should be sent to the President of the Swiss Federal Institute of Technology, E.T.H. CH 8006 Zurich, not later than March 31, 1974.

(434)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF ELECTRICAL AND ELECTRONIC ENGINEERING

Applications are invited for appointment as LECTURER in this Department. The salary scales range from £1,929 to £4,548. The commencing salary will depend on qualifications and experience. The appointment can be made from October, 1974, or from a later date by arrangement. Applicants should preferably have had industrial, research, or lecturing experience in communications, control or electronics. The person appointed will be expected to pursue research in addition to their teaching duties, for which facilities are available. The appointment will be held subject to the terms and conditions of the Grading and Salary Scheme, a copy of which is available on request. The successful applicant will be required to join the Federated Superannuation System for Universities, under the terms of which there is an annual premium of 15 per cent of the salary. The University will pay two-thirds of this premium and the member one-third. The University reserves the right to appoint to the post a person who has not submitted an application.

Further particulars and forms of application, returnable not later than February 11, are obtainable from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. (407)

UNIVERSITY OF BRITISH COLUMBIA

DEPARTMENT OF ZOOLOGY

Applications are invited from Ph.D.s with demonstrated ability in effective teaching first year biology; competence in any field of research. One, possibly two, assistant professors. Must be capable of making a positive contribution to teaching large class in introductory biology and to graduate programme: Salary range $10,000 to $15,000.

Apply to: Head, Department of Zoology, University of British Columbia, Vancouver, B.C. V6T 1W5. Appointment effective July 1, 1974, or by negotiation. Closing date: when position filled. (425)

UNIVERSITÄT ZU KÖLN, GERMANY INSTITUT FÜR GENETIK

PROTEIN CHEMIST

A post-doctoral research position for work in association with Dr. K. Beyreuther will be available immediately. The appointment will be for three years. The salary will be on the scale of about 27.000 DM per annum.

Applications, with curriculum vitae and the names of two referees, should be sent to Dr. K. Beyreuther, Institut für Genetik der Universität zu Köln, D5 Köln, 41, Weyertal 121, West Germany. (431)

SENIOR PETROLEUM GEOCHEMIST

A vacancy exists for a petroleum geochemist, with at least five years' experience, in the North Wales laboratories of Robertson Research International Limited. The laboratories are equipped to undertake a wide variety of analytical work, including high pressure liquid chromatography, gas chromatography, ESR spectrography and facilities also exist for spore colouration and vitrinite reflectance measurements. The position is permanent and pensionable.

Please apply to the Personnel Officer, Robertson Research International Limited, Llandudno LL30 1SA, U.K. (386)

AGRICULTURAL RESEARCH COUNCIL

APPOINTMENT OF FOUR SCIENTIFIC ADVISERS

The Agricultural Research Council invites applications for four posts at its Headquarters Office in London for Scientific Advisers to the Secretary of the Council concerned with (a) food science and (b) plant sciences (3 posts).

A good background of scientific studies and a proved record in research will be required. In addition, applicants should have studies in depth in one or more specialised fields related to the scope of their duties, preferably the following:

(a) **Scientific Adviser (Food Science)**
Microbiology.

(b) **Scientific Advisers (Plant Sciences)**
  (1) Plant Biochemistry/Cell Biology.
  (2) Genetics and Plant Breeding.
  (3) Crop Protection (Entomology: Plant Pathology).

Advisers are expected to aid in the formulation of agricultural research programmes and in their execution, and to advise on the staffing and equipping of agricultural research institutes and units. They are required to assist in the work of the Council and its Committees and Visiting Groups, and also with that of the Research Boards and Committees set up jointly by the Council, the Ministry of Agriculture, Fisheries and Food and Department of Agriculture and Fisheries for Scotland, by providing them with information and technical servicing. They have to establish and maintain regular working relationships with the Directors and scientific staff of the Research Institutes and Units and also with other organisations concerned with agricultural research and development, particularly with scientists in the agricultural departments. In addition to scientific expertise the work calls for the ability to express ideas clearly both orally and on paper.

The appointments will be at the level of Senior Principal Scientific Officer £5,725-£6,635 per annum according to experience. The salary scale, which is under review, is inclusive of Inner London Weighting. Superannuation under Civil Service type arrangements.

Further particulars about the posts may be obtained from the Establishment Officer, Agricultural Research Council, 160 Great Portland Street, London W1N 6DT, to whom applications should be sent by February 22, 1974. (438)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS

RESEARCH ASSISTANT/STUDENT

Applications are invited for the post of Research Assistant/Student for work on the dynamic stability of axisymmetric bodies at high Mach numbers. The work involves the development of a new type of aerodynamic facility and the exploitation of existing equipment for the measurement of dynamic stability derivatives.

Applicants should possess a good honours degree, or the equivalent, in a subject relevant to this research. The salary will depend on qualifications and experience and, in the case of a Research Assistant, will not exceed £1,750 per annum. The successful applicant will be expected to register for a higher degree.

Applications, including the names and addresses of two referees, should be addressed to The Deputy Secretary's Section, The University, Southampton SO9 5NH as soon as possible. Please quote reference number Na 182/R. (363)

FLATFORD MILL FIELD CENTRE

GRADUATES (females) wanted who would be interested in the post of Temporary Help (cooking) at Flatford Mill Field Centre whilst searching for permanent science appointments. Could attend interviews and leave at short notice if necessary. Would suit two friends.

Write: The Warden, Flatford Mill Field Centre, East Bergholt, Nr. Colchester, Essex, or telephone (reverse charges) East Bergholt (020-629) 283.

(397)

WELSH NATIONAL SCHOOL OF MEDICINE

(UNIVERSITY OF WALES)

Applications are invited for a

SCIENTIFIC OFFICER

in the Department of Haematology to work as one of a research team investigating neutrophil function in leukaemia. A background in the biological sciences required, but experience in haematology, whilst an advantage, is not essential. Preference may be given to candidates with a higher degree or, alternatively, opportunity to work for a higher degree may be provided for suitable candidates.

Salary scale on the Junior Scientific Officer scale £1,380-£2,112 or on the Scientific Officer scale £1,824-£2,517, according to qualifications.

Applications (no forms provided), accompanied by a curriculum vitae and the names and addresses of two referees, should reach the Registrar, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN, by February 28, 1974. (413)

UNIVERSITY OF MANCHESTER

There is a vacancy for a TECHNICIAN in the Electron Microscope Unit of the Botany and Zoology Departments. Applicants should have at least O.N.C. or equivalent qualification and preferably H.N.C. and have had 6 years' relevant experience including ultramicrotomy and other preparatory techniques. Salary scale £1,848 to £2,163 per annum.

Applications with full details of age, qualifications and previous experience should be sent to Dr R. Butler, Department of Botany, The University, Manchester M13 9PL as soon as possible. (359)

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF HUMAN BIOLOGY AND ANATOMY

SENIOR LECTURER/LECTURER required. Applicants must be medically qualified and capable of teaching topographical anatomy and neuroanatomy to medical and dental students. Research interests in the central or autonomic nervous systems desirable. Excellent research facilities, particularly in the fields of ultrastructure, histochemistry and autoradiography.

Salary scales: Senior Lecturer, £4,368-£5,496; Lecturer, £1,929-£4,548.

Applications (8 copies) with the names of three referees should be sent to the Registrar and Secretary by March 15. Further details may be obtained by writing informally to Professor R. Barer. (369)

THE UNIVERSITY COLLEGE OF WALES

ABERYSTWYTH

DEPARTMENT OF PHYSICS

Applications are invited for the post of SRC RESEARCH ASSOCIATE/SENIOR RESEARCH ASSOCIATE, to take part in a collaborative experimental programme between the Department of Physics at Aberystwyth and the Department of Electrical and Electronic Engineering at Liverpool. The programme is concerned with laboratory measurements of reactions occurring in the upper atmosphere and combines two experimental methods, the drift tube and the flowing afterglow. Most of the work will take place at Aberystwyth, but will involve travel between the two institutions. Appointment initially for one year, with possible extension to three years. Salary range: £1,929 to £2,718 per annum.

Application forms obtainable from the Registrar to whom they should be returned as soon as possible. (366)

Applications are invited from QUALIFIED CHEMICAL ENGINEERS, CHEMISTS, OR METALLURGISTS for research work in the field of high temperature electrochemistry involving steel—molten salt reactions and β-alumina type solid electrolytes. Two positions are envisaged, one at the graduate student level, for which applicants must be a Canadian citizen or a landed immigrant, leading to the degree of M.Sc. or Ph.D., the second at the postdoctoral fellow level. The work will be conducted in collaboration with the Atlantic Regional Laboratory of the National Research Council of Canada, Halifax, N.S. Remuneration will be at the rate of $4,000 to $4,500 per annum for the graduate student; $8,000 to $9,000 per annum for the postdoctoral fellow.

Please direct inquiries to:

Dr. D. R. Morris,
Department of Chemical Engineering,
University of New Brunswick,
Fredericton, N.B., Canada. (428)

DIRECTOR OF BASIC RESEARCH

Unique challenge to organize and direct a new "Center of Excellence" in basic aspects of modern cancer research. Program will be co-located with already-operating applied projects in viral oncology, chemical carcinogenesis, immunology and environmental control. Good scientific library, extensive experimental animal farm and other related support activities available.

Candidates should have a doctorate degree in one of the biomedical sciences and a minimum of 10 years' subsequent experience. Evidence must be presented of research originality, productivity and scientific leadership.

The successful candidate will be offered a liberal salary, attractive benefits and relocation expenses.

Letters of application, accompanied by a comprehensive curriculum vitae and complete bibliography, will be accepted until 15 March 1974. All correspondence should be addressed to:

Dr. Robert E. Stevenson
Chairman, Search Committee
Litton Bionetics, Inc.
Frederick Cancer Research Center
Post Office Box B
Frederick, Maryland 21701 U.S.A.

An Equal Opportunity Employer, M/F. (430)

ROTHAMSTED EXPERIMENTAL STATION

HARPENDEN, HERTS. AL5 2JQ

Applications are invited from suitably qualified scientists for the post of

HEAD OF THE PHYSICS DEPARTMENT

to succeed Dr H. L. Penman, FRS, who retires on March 31st 1974.

The Scientist appointed will be expected to develop lines of research relevant to agricultural physics, and more generally to provide scientific leadership to members of the Physics Department. It is hoped that the new Head will develop additional research programmes in soil physics.

Appointment in the grade of SPSO (£5,550–£6,460). Superannuation under FSSU with a non-pensionable allowance of 4½% of basic salary to offset personal contributions.

Applications, giving full details of career and naming two referees and quoting Reference 218 to the Secretary by 1st March, 1974. (379)

UNIVERSITY HOSPITAL OF SOUTH MANCHESTER

WITHINGTON HOSPITAL, MANCHESTER, M20 8LR

NATIONAL REFERENCE LABORATORY FOR ANTICOAGULANT CONTROL REAGENTS

Medical Laboratory Technician/Junior Medical Laboratory Technician required. Suitable for graduate with good I or II class honours degree in biochemistry, biology or physiology to train in technical procedures for routine blood coagulation work. Good opportunities for training for technical examinations in Medical Laboratory Technology. Salary £1,395–£2,292 per annum.

Applications in writing, naming two referees to Hospital Secretary. (14380). (375)

Nottingham Area Cytogenetics Service

Non-Medical Principal Scientific Officer

Applications are invited from cytogeneticists for the above appointment. The individual appointed will be responsible for a diagnostic chromosomal service which operates in conjunction with the newly-created Genetic Counselling Service in Nottingham.

The service will operate from the City Hospital, Nottingham, and laboratory accommodation is associated with the recently established University Departments of Obstetrics and Gynaecology, and of Child Health. There will be opportunities for association with the University departments. Applicants should have a higher degree and experience in a cytogenetics service unit. Salary in the range £3,516 to £4,599.

Application forms and further details can be obtained from the Secretary, Nottingham and District Hospital Management Committee, Sherwood Hospital, Hucknall Road, Nottingham NG5 1PD. (310)

WHATMAN BIOCHEMICALS LTD.

Process Technicians

Due to an expanding work load, we have vacancies for two Process Technicians in our Enzyme Plant at Maidstone.

The plant, itself, is fully equipped for growing bacteria and for extraction of enzymes from bacterial and other sources.

MICROBIOLOGY PROCESS TECHNICIAN

Applicants should have HNC or equivalent in a biological subject. Experience with Pilot scale fermenters or a good general background in microbiology would be an advantage.

ENZYME PROCESS TECHNICIAN

Applicants should have HNC or equivalent in a biological subject. Experience in enzymology and protein separation techniques would be an advantage.

Applicants for either position, qualified to ONC standard but with suitable experience, will be considered.

The work will be production oriented, but specific development projects will be undertaken under the direction of senior personnel.

Good salaries, 4 weeks' holiday, free life insurance, contributory pension scheme, luncheon vouchers.

Applications, giving details of qualifications and experience, to:

Personnel Manager, W. & R. Balston Ltd.,
Springfield Mill, Maidstone, Kent, ME14 2LE.

(423)

UNIVERSITY OF READING

DEPARTMENT OF MICROBIOLOGY

RESEARCH DEMONSTRATORS

Applications are invited for several posts of Research Demonstrator in the Department of Microbiology, commencing October 1, 1974. Applicants should have qualifications in Microbiology, Bacteriology, Biochemistry or related subjects. A demonstrator will be expected to demonstrate in practical classes up to eight hours per week during term and to engage in research for a higher degree (M.Phil., Ph.D.). Salary scale £1,047 by £51 to £1,149 per annum.

Further particulars from Dr. L. J. Zatman, Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ. (Ref. TN 1.) (393)

UNIVERSITY OF LONDON

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

COURTAULD INSTITUTE OF BIOCHEMISTRY

CLINICAL BIOCHEMIST (Research Assistant Grade) required in the Clinical Chemistry Laboratory, Courtauld Institute of Biochemistry. The post is suitable for a recent graduate to train in Clinical Chemistry with a view to taking the M.C.B. Salary according to age, qualifications and experience within the range £1,371 to £2,385 plus superannuation under the F.S.S.U.

Applications, including a curriculum vitae and the names and addresses of two referees to the Director, Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 5PR, by February 28, 1974. (401)

THE QUEEN'S UNIVERSITY OF BELFAST

LECTURESHIP IN PHYSIOLOGY

Applications are invited for a Lectureship in the Department of Physiology from April 1, 1974, or such other date as may be arranged. The successful applicant should have had experience in using histological techniques for research purposes and will assist in the teaching of histology to medical, dental and science students. The salary will be in the range £2,553 to £4,548 but in certain circumstances may rise to £5,496. If medically qualified, the successful applicant may be paid an additional salary on a sessional basis by the Eastern Health and Social Services Board for hospital services undertaken over and above University duties. Both salaries carry contributory pension rights under the F.S.S.U. The initial placing will depend on qualifications and experience.

Applications should be received by February 28, 1974. Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, BT7 1NN. (Please quote Ref. 74/N.) (374)

UNIVERSITY OF BRISTOL

DEPARTMENT OF SURGERY

Applications are invited for the post of

RESEARCH ASSOCIATE/ ASSISTANT

to study the immune responsiveness of patients with breast cancer. Candidates must possess a Ph.D. The post is annually renewable for two years and carries a salary of up to £2,553 per annum plus F.S.S.U. according to experience.

Applications, in writing, with the names of two referees, to Dr. M. O. Symes (from whom particulars may be obtained), The Medical School, University Walk, Bristol BS8 1TD, as soon as possible, please. (345)

UNIVERSITY OF MALAYA

FACULTY OF SCIENCE

Applications are invited for the posts of LECTURER/ASSISTANT LECTURER in the Departments listed below. Subject to academic suitability and experience, preference will be given to candidates who are competent in Bahasa Malaysia (Malay). Candidates should have at least a good honours degree with suitable teaching and research experience in addition to the special qualifications where indicated. For the Lectureships, a Doctorate is normally required.

DEPARTMENT OF GEOLOGY: 1 post of Lecturer/Assistant Lecturer to teach and carry out research in mineral evaluation and exploration techniques.

SCHOOL OF BIOLOGICAL SCIENCES: 1 post of Lecturer/Assistant Lecturer in the field of Invertebrate Zoology. Preference will be given to the candidate who possesses a good knowledge of the local invertebrate fauna especially the fauna of marine/ estuarine habitat.

Salary scales (approx. stg. equivs.): Lecturer £1,524 to £2,441 per annum; Assistant Lecturer £1,330 to £1,460 per annum. In addition the following allowances are payable: Variable Allowance £243 min., £649 max. per annum; Supplementary Housing Allowance £503 per annum and Medical benefits.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

The closing date for the receipt of applications is **February 18, 1974.** (360)

UNIVERSITY OF SYDNEY

CHAIR OF ANIMAL HUSBANDRY

Applications are invited for a Chair of Animal Husbandry (in the field of animal production). This vacancy has occurred following the resignation of Professor A. K. Lascelles, who occupied a Chair of Dairying in the Department of Animal Husbandry. Salary: $A19,102 per annum.

Applications, including curriculum vitae, list of publications, and names of three referees by 28 February, 1974, to the Registrar, University of Sydney, Sydney, New South Wales 2006, Australia, from whom further information available. Information also available from Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF. (416)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for the following:

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

PROFESSORIAL FELLOW: DEPARTMENT OF POPULATION BIOLOGY

The Department of Population Biology (Head: Professor B. John) is currently engaged in work on Australian insect populations. The successful applicant will be expected to develop a major line of research relating to the structure, the dynamics or the evolution of population systems. Only applicants with considerable research experience will be considered.

Closing date **February 15, 1974.**

THE NORTH AUSTRALIA RESEARCH UNIT

SENIOR RESEARCH FELLOWSHIPS/ RESEARCH FELLOWSHIPS

Appointees will be required to undertake interdisciplinary studies of contemporary social and economic conditions in northern Australia. Applicants will be invited to propose research projects falling within the general areas of enquiry which the University considers to be appropriate.

Applicants should preferably hold a doctoral qualification in an appropriate academic discipline and have had relevant research experience. The appointees will be based in Darwin where the Unit has its headquarters. Appointments are available from July 1974.

Appointment will be for two or three years, with a possible extension for continuing projects if the academic results achieved in the initial period are satisfactory to the University. A one-year appointment would be considered.

A District Allowance at the rate of $A700 per annum in the case of a married appointee or $A400 per annum in the case of a single appointee will be payable in addition to salary.

Closing date **February 28, 1974.**

FACULTY OF SCIENCE

CHAIR OF BIOCHEMISTRY

The Chair became vacant in November 1973 when Professor L. M. Birt resigned to take up the appointment of Vice-Chancellor Designate of Wollongong University College, New South Wales. It is hoped the appointee will be able to take up duty in 1974.

The Department of Biochemistry offers courses leading to the pass honours degree of Bachelor of Science and supervises students for the degrees of Master of Science and Doctor of Philosophy. Undergraduate teaching in biochemistry and, to a limited extent in microbiology begins in the second year of the Bachelor of Science course. Seven semester courses at second- and third-year level are offered. There are seven members of the academic staff (lecturers and above).

Closing date **March 25, 1974.**

Salaries: Salary for Professor will be $A19,102 per annum and for Professorial Fellow $A17,648 per annum. Salary on appointment to the other posts will be in accordance with qualifications and experience within the ranges: Senior Research Fellow $A12,778 to $A15,116 per annum (3 by $A390; 2 by $A389; 1 by $A390); Research Fellow $A8,698 to $A11,901 per annum (2 by $A641; 2 by $A640; 1 by $A641). Current exchange rates are approximately $A1; 65p; $US1.49.

Other conditions: Tenure: Professor and Professorial Fellow to retiring age (at present 65 years); Senior Research Fellow and Research Fellow normally for three years in the first instance with the possibility of extension to a maximum of five years.

Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra. Superannuation is on the F.S.S.U. pattern with supplementary benefits.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WO1H 0PF, for further particulars before applying. (361)

UNIVERSITY OF LEEDS

TECHNICIAN GRADE 5. HISTOLOGIST required in the Department of Physiology, Responsibilities would include the operation and management of a unit, working in close collaboration with research groups within the department, assist with the training of junior technicians in histological techniques, maintaining accurate records of work produced and control of stocks and forward requirements of the unit. Applicants must hold suitable qualifications for the post and have had a minimum of seven years' experience in general histological techniques. Salary is in the range of £2,007 to £2,382 per annum according to qualifications and experience.

Applications, stating age, qualifications and full details of experience together with the names and addresses of two referees should be addressed to Mr E. French, Department of Physiology, Medical Multipurpose Building, Mount Preston Street, Leeds 2. (378)

UNIVERSITY OF MALAWI

BUNDA COLLEGE OF AGRICULTURE

Applications are invited for the following posts tenable from September 1, 1974, for staff to teach at both degree and diploma levels:

1. Professor/Senior Lecturer in AGRICULTURAL ECONOMICS in the Rural Development Department

Applicants should have a higher degree in Agricultural Economics and at least five years' teaching and research experience in one or more of the following areas: development economics, marketing and farm management economics. The post carries with it the headship of the Rural Development Department which is responsible for teaching and research in Economics, Farm Management, Extension, Rural Sociology, Home Economics, Human Nutrition and Communication. Some administrative experience and a genuine interest in integrated rural development are desirable. Experience in socio-economic research for rural development would be an advantage.

2. Lecturer/Assistant Lecturer in AGRICULTURAL ECONOMICS in the Rural Development Department

Applicants should have a good honours degree in Agriculture or Economics with a higher degree or postgraduate qualifications and experience in Agricultural Economics. A strong interest and/or experience in integrated rural development is desirable.

3. Senior Lecturer/Lecturer in AGRICULTURAL EXTENSION in the Rural Development Department

Applicants should have a good honours degree in Agriculture and a higher degree or postgraduate qualification and experience in Agricultural Extension. Interest in the development of Extension Methods suitable for integrated rural development is desirable. Experience in agricultural extension in a developing country would be an advantage.

4. Professor/Senior Lecturer in LIVESTOCK PRODUCTION in the Livestock Production Department

Applicants should have a higher degree in Agriculture, Animal Science or Veterinary Science and at least five years' teaching and research experience in one or more of the following areas: nutrition, physiology, pasture production and management of livestock production systems. The post carries with it the headship of the Livestock Production Department and thus some administrative experience and a genuine interest in aiding the development of the livestock industry in Malawi is desirable.

5. Lecturer in LIVESTOCK PRODUCTION (2 posts) in the Livestock Production Department

Applicants should have a higher degree in Agriculture, Animal Science or Veterinary Science and teaching experience in one or more of the following areas: nutrition, physiology, pasture production and management or livestock production systems. The ability to teach vertebrate biology as applied to agriculture would also be desirable.

6. Lecturer/Assistant Lecturer in AGRICULTURAL CHEMISTRY/BIOCHEMISTRY in the Livestock Production Department

Applicants should have a good honours degree in Agricultural Chemistry/Biochemistry and appropriate postgraduate qualifications and experience. Ability to teach and research in some aspects of nutrition or physiology would be an advantage.

7. Lecturer/Assistant Lecturer in PLANT PATHOLOGY in the Crop Production Department

Applicants should have a good honours degree in the Biological Sciences or Agriculture and postgraduate qualifications and experience in plant pathology. The successful candidate will teach Plant Pathology as part of the Crop Protection course and Agricultural Biology and also develop practical research in Plant Pathology.

8. Lecturer/Assistant Lecturer in HORTICULTURE in the Crop Production Department

Applicants should have a good degree in Horticulture or Agriculture with postgraduate training and/or experience in Horticulture. Duties of the post include teaching horticultural subjects, the development of a fruit and vegetable demonstration unit at the College Farm and the initiation and development of practical research in horticulture relevant to the needs of Malawi.

Salary scales (including expatriate addition): Professor, £2,900 to £3,700 per annum; Senior Lecturer, £2,500 to £2,800 per annum; Lecturer, £1,340 to £2,250 per annum; Assistant Lecturer, £950 to £1,280 per annum, plus either a University addition of £300 per annum for Assistant Lecturers, £360 per annum for Lecturers, £500 per annum for Senior Lecturers or £680 per annum for Professors (taxable in Malawi) or supplementation (normally tax free) in appropriate cases by the British Government, in range £600 to £1,380 per annum (if married) or £300 to £1,100 per annum (if single). This latter supplementation is unlikely to be applied to appointments at Assistant Lecturer level. The British Government may also provide children's education allowances and holiday visit passages. Gratuity; superannuation scheme transferable with FSSU; family passages; various allowances; biennial overseas leave; housing. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be forwarded by air mail not later than February 28, 1974, to the Registrar, University of Malawi, University Office, PO Box 278, Zomba, Malawi. Applicants resident in UK should also send 1 copy to Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further particulars of the appointments are obtainable from either address. (456)

UNIVERSITY OF WESTERN ONTARIO

Applications are invited for the position of ASSISTANT or ASSOCIATE PROFESSOR. Responsibilities involve teaching undergraduate and gaduate students and conducting a research programme in biochemistry. Ph.D. and research experience in fields of physical biochemistry, membrane biology, or related areas will be given preference.

Send curriculum vitae and three references to Dr. W. C. McMurray, Secretary, Selection Committee, Department of Biochemistry, University of Western Ontario, London, Ontario, Canada N6A 3K7. (261)

UNIVERSITY OF LEICESTER

SENIOR LECTURER IN BIOCHEMISTRY

Applications are invited for the post of Senior Lecturer in the Department of Biochemistry. (Head of Department, Professor H. L. Kornberg, F.R.S.) This is a new post consequent upon the foundation of a Medical School at the University, and the person appointed will be concerned with teaching in the Faculty of Medicine and the Faculty of Science.

Initial salary, dependent on qualifications and experience, within the scale £4,368 to £5,496 a year, with F.S.S.U. membership.

Further particulars from the Registrar, to whom applications should be sent by March 4. (346)

UNIVERSITY OF MANCHESTER

RESEARCH FELLOW

DEPARTMENTS OF CHILD HEALTH AND COMMUNITY MEDICINE

Applications invited from science graduates with research experience involving the use of statistics, to participate in an epidemiological study of the 2,000 or so cases of children's cancer included in the Manchester Children's Tumour Registry. Three-year appointment funded by the National Institutes of Health, USA. Suitable training post for a career in cancer epidemiology. Starting salary not less than £1,929 p.a.

Further details and application forms (returnable by February 15) from the Registrar, The University, Manchester M13 9PL. Quote ref No 6/74/Na. (329)

SOUTHAMPTON UNIVERSITY HOSPITALS

RESEARCH TECHNICIAN in the Professorial Medical Unit, Royal South Hants Hospital, to work on aspects of immunology in relation to arthritis and leukaemia. An appropriate degree, H.N.C., A.I.M.L.T. or equivalent, is required.

Further details from Mr. R. Lloyd, Tel. 34288, Ext. 336.

Applications, giving details of experience and naming two referees, to Miss Jean Laughton, Personnel Department, Southampton University H.M.C., 119 Tremona Road, Southampton SO9 4XY. (415)

Classified Advertisements

CONDITIONS. All advertisements will only be accepted on the condition that the advertiser warrants that the advertisements do not in any way contravene the provisions of the Trade Descriptions Act 1968 and the Race Relations Act 1968. The Publisher also reserves the right to refuse, amend, withdraw or otherwise deal with all advertisements submitted to him at his absolute discretion and without explanation. All advertisements must comply with the British Code of Advertising Practice.

The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes.

Semi-displayed £3.60 per 10 mm. Minimum £7.20, each additional 2 mm 72p. Full page £230.00. Half page across £125.00. 30p is charged for the redirection of replies to advertisements with a box number.

ADVERTISEMENTS SHOULD BE ADDRESSED TO: T. G. Scott and Son, Limited, 1 Clement's Inn, London, WC2A 2ED. Telephone: 01-242 6264. Telegrams: Textualist, London, W.C.2.

APPOINTMENTS VACANT

ANGLO-EUROPEAN COLLEGE OF CHIROPRACTIC

Department of Pre-Clinical Studies

1. SENIOR LECTURER OR LECTURER (Grade II) in ANATOMY OR COMPARATIVE ANATOMY
2. SENIOR LECTURER OR LECTURER (Grade II) in MICROBIOLOGY AND/OR PATHOLOGY

Applications are invited for the above full-time posts to commence as soon as possible. Candidates must have suitable academic and/or professional qualifications and some teaching experience.

Initial salary dependent upon good qualifications and experience within the Burnham Scale ranges: £3,291-£3,655 for Senior Lecturer, £2,515-£3,243 for Lecturer Grade II. The posts are pensionable under the college's own scheme.

Detailed applications, including a curriculum vitae and naming two referees, should be sent to:

The Dean,
Anglo-European College of Chiropractic,
2 Cavendish Road,
Bournemouth,
Hampshire BH1 1RA. (262)

Research Unit on the Spatial Structure of Biopolymers

A research unit on the spatial structure of ribosomes and their components is to be established at the Max-Planck-Institut für Molekulare Genetik in Berlin-Dahlem, with the financial support of the Deutsche Forschungsgemeinschaft.

Applications are invited for the positions of the head of this research unit and for scientists working in the unit. The annual salary for the head will be approximately 45-50,000 DM and for the scientists 32-42,000 DM. The initial appointment will be for three years with the possibility of extension.

Applicants should have experience in one of the following fields: Crystallization of proteins or nucleic acids, X-ray crystallography of biopolymers, chemistry of proteins or nucleic acids. It is not necessary for the applicants to be German.

Applications should be sent before March 30, 1974, to Professor H. G. Wittmann, Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 63-73, D-1000 Berlin 33, Germany.

(427)

PHYSICAL PHARMACY

The role of our Pharmaceutical R & D department is to devise a formulation and process for the preparation of finished dosage forms from discovery chemistry in both the UK and the US and to demonstrate that these procedures will consistently result in stable, efficacious products.

Two pharmacy graduates are now needed to meet planned expansion needs of our Physical Pharmacy section which investigates the problems of stability and bio-availability of new drugs.

For one appointment preference will be given to PhDs with research experience in physical or colloid chemistry. Ideally we seek those with experience in powder technology or reaction kinetics.

The Department offers a scientifically stimulating environment with excellent technical support, laboratory equipment and scientific services. There is also ample opportunity for interaction with scientists of many disciplines.

Our conditions of employment include free pension and death benefit scheme, assistance with removal expenses (where appropriate), four weeks annual holiday, etc.

Pfizer

Our location is rural/coastal at Sandwich, Kent.

Applications, giving brief details of age and experience, should be addressed to:

D. W. Sells, Personnel Manager,
Pfizer Central Research,
Sandwich, Kent, CT13 9NJ.

(452)

UNIVERSITY OF OXFORD

DEPARTMENT OF BIOCHEMISTRY

GENETICS LABORATORY

It is proposed to appoint a DEPARTMENTAL DEMONSTRATOR IN GENETICS from October 1 next, the salary to be up to £2,283 per annum according to qualifications and experience, together with membership of F.S.S.U. (or equivalent—under review). The appointment is for a period not exceeding 3 years in the first instance and may be renewable for a further period up to a total of 6 years.

Other things being equal, preference may be given to candidates working in, or interested in working in, the field of the genetics and biochemistry of animal cells.

The successful candidate will be expected to take part in Departmental teaching and to conduct his own research.

Applications (three copies) including the names of two referees should be sent to the Administrator, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, not later than March 11, 1974.

(390)

SCOTTISH HORTICULTURAL RESEARCH INSTITUTE

INVERGOWRIE, DUNDEE DD2 5DA

PLANT BREEDER (SO/HSO)

A plant breeder is required to participate with a team engaged in breeding raspberries and blackberries.

Appointment in the grade of Scientific Officer (£1,590 to £2,177 per annum) or Higher Scientific Officer (£2,076 to £2,667 per annum) depending on experience. F.S.S.U. with 4½ per cent additional allowance to offset contributions. Candidates should have at least an Upper 2nd Class Honours degree in Botany or Agricultural Botany.

Further particulars are available from the Secretary, to whom applications giving details of age, education and experience, together with the names of two referees, should be sent by February 28. (398)

UNITED SHEFFIELD HOSPITALS

NON-MEDICAL SCIENTIFIC OFFICER

required until October, 1975, to take part in a research project investigating into the Excretion of Sulphated Corticosteroids in Breast Cancer.

Applicants should possess a first or second class Honours degree

Whitley Council Terms and Conditions of Service apply. Salary £1,680-£2,112 p.a.

Applications, stating age, qualifications and experience and naming two referees, to the Acting Chief Administrative Officer, United Sheffield Hospitals, 10 Beech Hill Road, Sheffield S10 2RZ. Further details supplied on request. Closing date for applications: February 16, 1974. (440)

A New Year –
A New Journal: Cell

January 1st was not only the first day of a new year—but also the publication date of the first issue of **Cell**, a new monthly journal of cell and viral biology from The MIT Press. Edited by Benjamin Lewin, formerly the editor of *Nature New Biology,* **Cell** provides a wide-range journal publishing articles of general interest in molecular biology, cell biology, tumor virology and immunology, genetic development and other related areas. Papers are refereed as rapidly as possible and those accepted will be published within twelve weeks.

Cell also includes an extensive review section. In addition to those reviews with the traditional role of discussing a field in some depth, short reviews give conceptual accounts of recent advances in areas of cellular biology, for the benefit of researchers in other areas. Book reviews and discussions of meetings are also included.

The January issue of Cell includes the following articles:

Genetically directed preferential X-activation seen in mice: U. Drews, S. R. Blecher, D. A. Owen and S. Ohno

Ouabain-resistant mutants of mouse and hamster cells in culture: R. M. Baker, D. M. Brunette, R. Mankovitz, L. H. Thompson, G. F. Whitmore, L. Siminovitch and J. E. Till

A uridine kinase-deficient mutant of 3T3 and a selective method for cells containing the enzyme: L. Medrano and H. Green

SpoT, a new genetic locus involved in the stringent response in E. coli: T. Laffler and J. Gallant

The messenger-like poly-A containing RNA species from the mitochondria of mammals and insects: M. Hirsch, A. Spradling and S. Penman

Existence of methylated messenger RNA in mouse L cells: R. P. Perry and D. E. Kelley

Arrangement of specific oligonucleotides within poly-A terminated Hn RNA molecules: G. R. Molloy, W. R. Jelinek, M. Salditt and J. E. Darnell

Mixed splenocyte cultures and graft versus host reactions selectively induce an "S-tropic" murine type C virus: C. J. Sherr, M. M. Lieber and G. J. Todaro

Studies on the levels of cyclic AMP in cells transformed by wild type and temperature sensitive Kirsten sarcoma virus: R. A. Carchman, G. S. Johnson, I. Pastan and E. M. Scolnick

The February issue of Cell will include the following articles and reviews:

Viral Hepatitis, the B Antigen and Liver Cancer: A. J. Zuckerman

Integration and Excision of Phage Lambda: M. Gottesman

GUG and UUG are Initiation Codons In Vivo: J. H. Miller

The Molecular Biology of Tumour Viruses, edited by John Tooze: B. Mahy

Altered Metabolism of the Guanosine Tetraphosphate, ppGpp, in Mutants of E. coli: G. Stamminger and R. A. Lazzarini

Continuous Production of Radiation Leukemia Virus in C57BL Thymoma Tissue Culture Lines: Purification of the Leukemogenic Virus: M. Haas

Changes in RNA in Relation to Growth of the Fibroblast. I. Amounts of mRNA, rRNA and tRNA in Resting and Growing cells: L. Johnson, H. Abelson, H. Green and S. Penman

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matters arising

Rapid fluctuations of large volume astronomical sources

SIR,—The argument that the characteristic fluctuation time of an astronomical source implies that the source be smaller than a characteristic dimension is often quoted without adequate discretion, particularly concerning QSOs[1] and pulsars. That argument is actually valid only for incoherent generation of radiation and since non-thermal radiation mechanisms are strongly suspected the validity of the argument must also be suspected.

As a simple counter-example I take the case of a rapidly fluctuating star situated in a large absorbing cloud. For an external observer the star appears to have unaltered dimensions and fluctuations, but lower brightness temperature, compared to if the cloud were absent. Now if the cloud were to have negative absorption (maser amplification) the external observer would still see the same dimensions and fluctuations, but higher brightness temperature, again compared to if the cloud were absent. The observer would not see the cloud itself even though the power generation could occur throughout the full volume of the cloud.

Such a mechanism could clearly alleviate the power density dilemma of quasars even at cosmologic distances. QSOs would be able to fluctuate rapidly and also appear small even though the power generating volume were large.

It turns out that such an amplifying cloud could also alleviate the second principal dilemma for cosmological QSOs; their apparent connections with peculiar local galaxies. Rozyczka[2] has mentioned three possible explanations for those connections. They may be: (1) real; (2) by chance; or (3) caused by the amplification of radiation in the vicinity of the peculiar local galaxies. Large amplifying clouds, this time surrounding galaxies, again might conciliate puzzling observations.

My purpose here is not to advocate amplifying clouds, for they raise problems as serious as those they solve. The source of energy and the required pumping and amplification mechanisms would pose enormous difficulties. (The energy source is actually a serious problem for almost any explanation.) The purpose is rather to provide a reminder that thorough and meticulous logic must be exercised during deductions if the conclusions are to be valid.

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* On leave from the Smithsonian Astrophysical Observatory.

[1] Burbidge, G. R., *Nature phys. Sci.*, **246,** 17 (1973).

[2] Rozyczka, M., *Acta Astr.*, **23,** 233 (1973).

Announcements

Appointments

B. S. Hartley of the **Medical Research Council Laboratory of Molecular Biology at Cambridge** has been appointed to the chair of biochemistry **at Imperial College,** London.

G. E. Eden has been appointed to take charge of the **Water Pollution Research Laboratory,** Stevenage.

W. G. Crewther has been appointed chief for the **CSIRO Division of Protein Chemistry.**

International Meetings

February 12, **High Performance Liquid Chromatography: A forum for the Exchange of Experience with Apparatus** (The Society for Analytical Chemistry, Analytical Division, Chemical Society, 9/10 Saville Row, London W1X 1AF)

February 13, **Fundamentals of Remote Sensing** (General Secretary of the Remote Sensing Society, Dr W. G. Collins, Reader in Remote Sensing, Remote Sensing Unit, University of Aston, Gosta Green, Birmingham, B47ET)

February 13–15, **Biological effects of Electromagnetic Radiation** (Public Relations, The New York Academy of Sciences, 2 East 63rd Street, New York 10021)

February 19, **High Performance Liquid Chromatography** (The Society for Analytical Chemistry, Analytical Division, Chemical Society, 9/10 Saville Row, London W1x 1AF)

February 20, **Automatic Turbidimetry** (The Society for Analytical Chemistry, Analytical Division, Chemical Society, 9/10 Saville Row, London W1X 1AF)

February 20, **The Rational of Control of Sterile Areas** (The Society for Analytical Chemistry, Analytical Division, Chemical Society, 9/10 Saville Row, London W1X 1AF)

February 20–21, **The origin of Cosmic Radiation** (The Executive Secretary, The Royal Society, 6, Carlton House Terrace, London SW1Y 5AG)

February 27, **Secondary Steelmaking** (Meetings Secretary, Institution of Metallurgists, Northway House, Whetstone, London N20 9LW)

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Greater London Council Scientific Branch. Annual Report of the Scientific Adviser 1972. Pp. 136. (London: Greater London Council, 1973.) £4. [71

British and Continental Progress in Water Pollution Control. (Sixth Public Health Engineering Conference, held in the Department of Civil Engineering, Loughborough University of Technology, January 1973.) Edited by John Pickford.. Pp. 86. (Loughborough: John Pickford, Department of Civil Engineering, University of Technology, 1974.) £3. [71

The Astronomer as Natural Philosopher. By Professor A. H. Cook. (An Inaugural Lecture.) Pp. 36. (London: Cambridge University Press, 1973.) 45p net; $1.75. [81

ARC Index of Agriculture and Food Research. Pp. viii + 132. (London: Agricultural Research Council, 1973.) £1. [81

Getting Down to Fundamentals—the Particle Spectrum. By Professor I. Butterworth. Inaugural Lecture, November 14, 1972.) Pp. 67–82. 45p. The Synthesis of Dynamical Systems. By Professor D. Q. Mayne. Pp. 83–102. 45p. Time, Gravitation and the Universe: The Evolution of Relativistic Theories. By Professor G. J. Whitrow. (Inaugural Lecture, May 22, 1973.) Pp. 103–126. 45p. (London: Imperial College of Science and Technology, University of London, 1973.) [81

Potatoes—Consumer Buying Behaviour and Attitudes during 1972–73. (A Report of a Survey conducted for the Potato Marketing Board by Research Bureau Limited.) Pp. 47. (London: Potato Marketing Board, 50 Hans Crescent, SW1.) [111

Cranfield Institute of Technology. Annual Report 1973. Pp. 14. Cranfield Bedford: Cranfield Institute of Technology, 1974.) [111

Agricultural Research Council Animal Breeding Research Organisation—Report, January 1974. Pp. 56. (Edinburgh: ARB, Animal Breeding Research Organisation, 1974.) 40 p.

Desert landforms

Geomorphology in Deserts. By Ronald U. Cooke and Andrew Warren. Pp. 374. 80 plates. (Batsford: London, August 1973.) £5.50.

LANDFORMS in deserts, unobscured by vegetation and constituting the scenery, are remarkable for their abruptness and orderliness. Until recently there were few books in English concerned mainly with desert geomorphology. *Deserts of the World* by W. G. McGinnies *et al.* (Office of Arid Lands, University of Arizona, 1968) contains only one chapter on geomorphology; K. W. Glennie's *Desert Sedimentary Environments* (1970) is more specialised, but the volume under review rarely overlaps it.

The field experience of the two authors has been complementary. Cooke has published on pediments, gullies and desert pavements in North and South America. Warren has worked on soils and dunes in Pakistan and the Sahara.

Their book is in four parts. In the first the diversity of desert landforms is emphasised and different approaches to their study are assessed. The concepts of W. M. Davis are treated rather unsympathetically but the wonder is that his arid cycle has been treated so seriously; he obviously regarded it as a sighting shot. The authors themselves adopt a more fashionable systems approach, one which I think is not ideally suited to a subject in which discontinuity of operations is so marked.

Most of the world's deserts, even the southern Sahara, were not truly arid as recently as 5,000 to 15,000 years ago and before that their climates, at least through the Quaternary, fluctuated markedly. Such instability seems to me to present difficulties for a systems approach to the study of the resultant landforms. I am not convinced that the return period concept releases geomorphologists from the necessity of concerning themselves with paleoclimatology; the two must go together.

The soil surface sytem is the subject of part 2. It brings together very usefully a great deal of recent literature on desert soils. Part 3 deals with integrated patterns of change on slopes and in channels, and with features such as pediments, fans and playas. There should be more photographs illustrating these features. Pediments remain enigmatic; as with so many forms that attract attention, their vital statistics characterise without necessarily revealing their underlying nature.

In part 4, "Aeolian Systems," a couple of equations have somehow been missed out and there are a number of minor slips, but the section on dune patterns is very stimulating. It draws on the work of the late Ian Wilson and I hope that Warren and others will pursue the subject further with the cooperation of aerodynamicists.

A. T. GROVE

All immunology

Handbook of Experimental Immunology. Edited by D. M. Weir. Second edition. Pp. 350. (Blackwell Scientific: Oxford and London, 1973.) £22.50.

THE second edition of the handbook weighs 3,000 g, an indication both of the editor's assiduity and of the growth of immunology (37% increase in weight over the previous edition!). The utility of this monster will be revealed with time and the passage of many toil-stained fingers but even now deficiencies are clear.

The three sections of the book are labelled "Immunochemistry", "Cellular Immunology" and "Applied Immunology." Individual chapters are written in the main by people acknowledged by their peers as experts in particular fields. But it is already nearly two years out of date which in cellular immunology would by many be judged a crime. Even if it were up to date it is not comprehensive and this is particularly true of the applied immunology section. Hardly any of the current attempts to apply cellular immunological methods, experimentally, in clinical contexts or in studies of experimental infection are documented in the handbook; there is very little on application of the methods of rosette-forming cells, anti-theta analysis, cell electrophoresis, velocity or density sedimentation of lymphocytes and general surface antigens of lymphoid cells. Many methods are given in such detail as to duplicate other parts of the book. The many recipes for PBS phosphate-buffered saline are a case in point. Judicious editing might have avoided quite so much overlap and thereby reduced the expense. The index is the only form of cross-referencing between chapters which otherwise, and perhaps inevitably, seem to have been written in isolation.

On the good side are the very many chapters which do beautifully exemplify the contemporary immunological scene. The illustrations are good and clear almost throughout the book. There is a wealth of the folk-lore of immunological method honestly set down. The handbook is an heroic effort but its high price and the speed of change in immunology combine to make it as far as its cellular immunological parts are concerned a white elephant.

A. J. S. DAVIES

Black holes expand minds

Black Holes: The End of the Universe? by John Taylor. Pp. 174. (Souvenir: London, October 1973.) £2.50.

IT is difficult to see this book as other than an attempt to pre-empt future science fiction writers by attaching a scare story of death and destruction to every aspect of black holes.

The book is glued together with long tedious passages discussing the significance of black holes to mankind and the author uses the first forty pages to make general remarks about the state of the world, religion, precognition, astrology and so on. He announces that America has cut oil supplied to airlines, that Heitsi-ebib, a god of the Hottentots, died several times, that when Huxley and Wilberforce argued on evolution Lady Brewster fainted, and many other gratuitous scraps.

Then black holes are introduced. The chapter starts "None of us wants to die, but death is with us whether we like it or not; sadly we have not yet discovered how to avoid it". For the next hundred pages the author wanders through various properties of black holes with an unerring eye for the spine chilling. "The pulverized mess that was [the astronaut's] remains would be crushed ever smaller". "The bones and muscles of his body will be elongated beyond breaking point". This is as unhelpful as would be a ceaseless emphasis in a book on plate tectonics, on the horrid death to be expected if one stayed on an oceanic plate at a subduction zone.

It would be difficult for a layman to appreciate whether the chapter on extracting energy is anything other than pure tongue-in-cheek. It would be equally difficult for him to grasp some of the concepts which are introduced in such an off-hand way—in phrases such as "Time passes very, very slowly for the first few billionths of a second (of the universe)" or "Light possesses energy and so has mass". Professor Taylor declares it "certain" that there is life elsewhere in the Universe and goes on to guess what it is like. The relevance of this to black holes is re-revealed in breathtaking manner when the biblical legends of Satan are interpreted as God casting Satan into a black hole (the "fiery furnace"). The Devil's horns "could indicate antennae" confirming that he came from distant parts of the Galaxy.

The author says in the concluding paragraph of the book that the black hole Universe affords "great opportunities to expand our vision of the world". Just so.

DAVID DAVIES

Enhancing immunity

Immunopotentiation (Ciba foundation Symposium 18, New Series.) Pp. ix+335. (Elsevier/Excerpta Medica/North-Holland, Associated Scientific Publishers: Amsterdam, London and New York, 1973). Dfl. 47.50; $18.30.

This symposium deals with the effects of substances (adjuvants) which can stimulate the immune response to unrelated antigens. This topic is of obvious interest as far as control of the immune response is concerned, but becomes of major importance because of its clinical significance. Adjuvants can help in cancer therapy, and the proceedings summarise the situation in this area, and suggest mechanisms whereby adjuvants may be acting.

Convincing evidence is presented of the efficacy of immunotherapy for cancer treatment in laboratory animals and human disease. A rationale for the use of adjuvants in some human leukaemias is clearly advanced by Mathé. If chemotherapy is first used to reduce the tumour mass, immunotherapy involving the injection of BCG and killed tumour cells can be strikingly successful in prolonging remission of the disease.

It seems unlikely that the agents useful in cancer therapy act on the immune system, mainly because they are also powerful adjuvants for many well characterised immune responses. The effects of adjuvants on these responses is also dealt with. Some participants suggest that adjuvants may potentiate the trapping of lymphocytes in lymph nodes or spleen and improve cell interactions. Others favour the view that adjuvants directly stimulate cells involved in immune responses. In this regard the B lymphocyte, T lymphocyte, macrophage triangle is dominant, and effects on all three cell types are proposed. Perhaps one can generalise to say that macrophages certainly seem to be strongly involved in adjuvant action, and that different adjuvants can have effects biased towards B or T lymphocyte responses. Some can even potentiate one type of immune response and suppress another.

As the proceedings make clear, what is needed is an understanding of the control mechanisms involved, and hopefully the discovery of methods of selectively stimulating or suppressing the different types of immune response.

Ciba Symposium 18 should be of great interest to those studying the immune response or cancer therapy. Immunopotentiation is a confusing subject, and the presentation of the 1973 state of play is of great value; the discussions of the participants are particularly useful. It is clear that there is work to be done, and anyone reading the volume should find a few ideas to begin with.

A. F. Williams

Creative stimulus

Population Growth: Anthropological Implications. Edited by Brian Spooner. Pp. xxvii + 425. (MIT: Cambridge Massachusetts and London, 1973.) $15; £6.75.

This collection of papers considers various anthropological problems in the light of the population theories of Ester Boserup[1]. She considers that human population growth acts as an independent variable in the development of culture and society. Population growth is not inexorably limited by the level of resources through the agency of 'misery and vice' but instead stimulates invention to generate new resources or new techniques for exploiting old resources.

There are seventeen papers in all. The first six are concerned with archaeological evidence. A mass of archaeological data is presented for Mesoamerica, Mesopotamia and Egypt. Sometimes this is accompanied by population density estimates, sometimes not. In most of these papers the relationship of population change, resource exploitation and economic theory is treated very much as a postscript.

Bronson's paper "Farm Labour and the Evolution of Food Production" is one of the few where theory is discussed at length. According to Boserup, farmers will always prefer easy extensive cultivation (like shifting cultivation) until they are forced to intensify methods by population pressure. Furthermore, farmers will revert to shifting cultivation if population size falls. But where population is known to have fallen, as in Europe after Rome's fall, no such agricultural change is seen. Bronson also complains that the more elaborate stages of agriculture should not necessarily be regarded as being more 'adaptive' than the simpler ones, especially when the latter are still represented in present-day economies. If Boserup were correct, shifting agriculture would be extinct, whereas it is still widely practiced in areas which could support more intensive systems.

Most of the rest of the book deals with present-day populations from the viewpoint of social anthropology. One paper considers the importance of religious organisation and suzerainty in achieving political centralisation. Another describes conditions in Tibet which support Boserup's theory through its obverse: despite a superabundance of resources a population declines for non-economic reasons and migration takes place into the area of its most extensive deployment; high altitude nomadic pastoralism. One of the most interesting papers presents calculations to show how the problems of carrying children force the nomadic Kung bushmen to prolong the interval between births; a type of problem not dealt with in Boserup's economic formulations.

As a discussion of Boserup's theory in relation to anthropology this book is a disappointment, and the title is certainly misleading. The archaeological data are doubtless interesting in themselves and some of the population estimates may be useful. But demographic data from archaeological and ancient literary sources are unreliable and may always remain so. Associations may be established between past population growth and technical change, but Boserup's theory seems to require population change to occur first and, of course, for the postulated mechanism to be responsible. This would be difficult enough to establish in a modern population and it is not clear how the necessarily less detailed information from archaeology could hope to validate such a theory.

But most of the papers on modern populations are also unable to demonstrate the importance of the demographic factor, partly through their lamentable lack of numerical information. In many cases no demographic data are quoted, population is rarely mentioned and resources are not evaluated.

It is very surprising that no reference is made to historical European populations whose demography and economics are much better known than those of ancient Egypt or Mesopotamia. Exogenous technical innovations, which have had profound demographic consequences, might also have been germane to these discussions. But the introduction of the potato to Europe, or of European sanitary and medical knowledge to the Third World, are nowhere mentioned.

It seems that many archaeologists and anthropologists need to know more demography. The difficulty of establishing a trustworthy case for population change does not seem to be appreciated, the impossibility of sustained population growth or decline for more than a short time is not realised, and the views of Malthus and other theorists (often thought to be in opposition to Boserup) are widely misunderstood by the contributors to this book.

D. A. Coleman

[1] Boserup, E., *The Conditions of Agricultural Growth: The economics of Agricultural change under Population Pressure* (Aldine, Chicago, 1965)

of astrophysics has made many people look forward to a text on stellar structure from the pen of Professor Swihart. Such a text has now appeared in the form of a companion to his earlier *Basic Physics of Stellar Atmospheres*, and as an introduction into the field of stellar structure the new book can be quite as strongly recommended as Professor Swihart's earlier writings. This book is a short introductory text, but one which contains all the essential points. There are discussions of conditions of gas in thermodynamic equilibrium, of polytropes, of energy generation and transport, of radiative transfer and opacity. There is a final chapter devoted to a discussion of theoretical stellar models and to their evolutionary changes as the result of changes in their chemical composition. Present problems and discrepancies are as clearly stated as the remarkable overall agreement between theory and observation which exists in this field.

As in his earlier book the author has again found room in an appendix for a dozen problems whose solution will test a student's understanding of the text. The only change which I would like to suggest for the next edition of this most useful book is the adoption of the international system of units.

H. A. Brück

Chemicals in soil

Organic Chemicals in the Soil Environment. Edited by Cleve A. I. Goring and John W. Hamaker. (Books on Soils and the Environment Series.) Vol. 1, pp. xii+440; vol. 2, pp. xii+441–968. (Dekker: New York, August and September 1972.) Vol. 1, $24.50; vol. 2, $26.50.

Chemical pollution of the environment is highly emotive, but solid facts are often lacking, so the publication of this tremendous work is certainly timely. Most people understand that the soils which mantle the Earth are a basic resource to be carefully husbanded in order to increase the world output of food and other raw materials. To achieve this end man has come to depend more and more on the application of fertilisers through foliar sprays or, more usually, through the roots by way of the soil; on optimising the water supply through the soil; and on minimising biological competition from weeds, pests and diseases through foliar or soil applications of crop protection chemicals.

This work comprises thirteen chapters grouped in four parts dealing respectively in vol. 1 with "The Soil Environment" (one chapter) and "Physiochemical Relationships of Organic Chemicals in Soil" (five chapters); and in vol. 2 with "Action and Fate of Organic Chemicals in Soil" (five chapters) and "Environmental Effects of Organic Chemicals" (two chapters).

The editors head agricultural research at the Ag-Organics Department of the Dow Chemical Company in California. Both are men of distinguished research and organising ability; they have themselves contributed four chapters and enlisted the able help of colleagues, Drs Brandt and Meikle, to write two others, so that about half the total work emanates from the Dow Company. The remaining authors are all highly competent researchers and writers, including Dr Edwards from Rothamsted Experimental Station and Dr Thomas of Birmingham University; the net result is a well balanced text.

Chapter 1 by J. L. Ahlrichs of Purdue University is a comprehensive introductory account of macrocharacters of soils as they occur naturally and of the microcharacters of the soil solid, liquid, gaseous and living phases including some references to enzyme activity: some readers may be puzzled by the USDA "7th approximation" alternative names for soil horizons.

Chapter 2 by J. W. Hamaker and J. M. Thomson is a very detailed and valuable treatment of surface absorption principles. Chapter 3 by R. W. Meikle deals mainly with the biochemical mechanisms of decomposition, in soil systems, of the synthetic organic chemicals used for crop protection and includes a discussion of the "permease" concepts of active transport across cell membranes which those not familiar with these ideas will appreciate. Chapters 4 and 5 by Hamaker are concerned respectively with detailed reaction kinetics in relation to conditions in the soil environment and with diffusion and volatilisation in the gaseous phase. Chapter 6 by Professor Letey and J. Oddson continues the theme of movement in the soil system, chiefly through mass flow in aqueous solution.

Chapters 7, 8 and 9 are respectively excellent reviews of herbicides and plant growth regulators (R. P. Upchurch), insecticides (C. A. Edwards), and fumigants, fungicides and nematicides (C. A. Goring). Then comes a change from the dominant crop protection materials to a consideration by J. D. Hauck in chapter 10 of synthetic slow release fertilisers and fertiliser amendments, and in chapter 11 of soil physical property modifiers by G. H. Brandt.

The final part is devoted to a chapter by Professor Martin on side effects of organic chemicals on soil properties and plant growth, and one on Goring's viewpoint of the impact of agricultural chemicals on the environment with some valuable data on input levels, accumulations and toxicity levels.

Every chapter is well written and illustrated, with very few typographical errors. Each concludes with numerous references and at the end of vol. 2 there is a very useful list of the full chemical names against each of the common or temporary names quoted, followed by comprehensive author and subject indices. Though the books are costly for private pockets they are highly recommended to all who have a professional interest in the subject and for library acquisition. The authors and editors are to be congratulated on bringing together in comprehensive, readable form a mass of detailed information, much of which has hitherto been inaccessible to the average inquirer. Furthermore, it is infused with a good many stimulating ideas and comments.

J. Tinsley

Crystal physics

Tensors and Group Theory for the Physical Properties of Crystals. By W. A. Wooster. Pp. x+344. (Clarendon: Oxford; Oxford University: London, August 1973.) £7.

This book may be regarded as an expanded and modernised version of Dr Wooster's well known *Crystal Physics* which was first published in 1938. Its principal objective is "to enable students to grasp the essential techniques of dealing with tensors and group theory as they are applied to crystals". Numerous support exercises are interwoven with the text to which detailed answers are provided. The author's mastery of crystal symmetry and crystal physics comes through on almost every page, but whether he has achieved his stated objective remains open to question.

Tensors and groups are introduced in a piecemeal fashion without being built up into any coherent body of theory. This approach might well give the student deeper insight into the meaning of the symbolism, as Wooster points out in his introduction, but a heavy price must be paid. The theory of the crystallographic groups cannot really be understood without reference to the basic finite groups, including particularly the cyclic, dihedral and tetrahedral groups. Any attempt to bypass these leads inevitably to lacklustre multiplication tables and unduly complicates the determination of the conjugation classes. It also precludes the recognition of useful isomorphisms between apparently different groups. Similarly the discussion of polar and axial vectors suffers from an inadequate mathematical framework. It is no paradox to suggest that a dash more of mathematics could have reduced the length of this book and improved its clarity. But, despite these criticisms, it makes a refreshing change from the over-mathematical texts which hardly make any contact with physical ideas, and it contains a tremendous amount of valuable information. M. A. Jaswon imagine. Apart from a few small changes, the first 330 pages are word for word copies of the earlier work (the use of a pair of scissors to rearrange some of the sections should not confuse the comparison). The claim on the dust jacket that this material has not been available previously in book form took me by surprise. Let me suppose, however, that the bookseller has been persuaded to divide the spine at page 330 and has accepted back also seven appendices; this leaves £4.25 worth of new material at a *pro rata* rate per page. The new material consists of four chapters, two on the quantum theory of damping and two on the theory of the laser.

The author's approach to these matters is based on the idea, explicitly state on the dust jacket, that *c*-number equations are more easily solved than quantum equations. The earlier book was devoted to the elaboration of the technique of the Wigner distribution function and related functions for converting quantum mechanical equations into apparently classical equations. The idea is not at all proved (or even discussed further in the text) and, in fact, a quick thumb through these chapters would indicate to a trained mathematical physicist that the opposite might be the case. This is particularly true of quantum optics, where spontaneous emission, which is the key to quantum noise, is trivially easy to identify in the quantum equations but is deeply buried (finally even to disappear in the classical limit) in the equations of classical form.

There is no accounting for taste, however, and ignoring the strictures above, Professor Louisell has done a very thorough job within his brief. The earlier chapter 3 is augmented by five new "ordering" theorems and the detailed mechanisms of the damping of a system by interaction with a reservoir are worked through in the first of the chapters on quantum damping. In the second of these chapters, damping is studied from the point of view of Langevin forces which are derived consistently with quantum commutation requirements. No contact, unfortunately, is made with modern Langevin statistical mathods such as those of Kubo and Mori. Finally, the theory is expressed in *c*-number form by use of appropriate ordered distributions. This is material familiar to those who have studied the methods of Lax and his colleagues over the years and is convenient to have in a form other than the original papers or summer-school notes.

Of the two chapters on the laser, the first discusses the semiclassical theory after Lamb, and the second statistical properties following the Fokker–Planck treatment due independently to the schools of Lax and of Haken. No great depth is achieved and I could trace no mention in the whole book of anything so mundane as an experiment.

At £14 this work will not appeal to the general optical physicist. The theoretical specialist, who will have the earlier volume, will be loath to spend this sum for so little new material.

E. R. PIKE

Atmospheric dynamics

Atmospheric Energetics. By Jacques Van Mieghem. Pp. ix+306. (Clarendon: Oxford; Oxford University: London, April 1973.) £7.50.

THIS treatise is based on the author's lectures on atmospheric dynamics at the University of Brussels and at the seminar of the Department of Aerology of the Royal Meteorological Institute of Belgium. Part 1, comprising the first seven chapters, is devoted to the basic energy equations for laminar and turbulent flow. There then follow chapters on the energetics of small-scale forced and free convection.

The main emphasis of the book lies, however, in the treatment of the energetics of large-scale atmospheric systems and the author develops this topic extensively, reflecting his own considerable original contributions in this field. Chapter 11 considers the generation of eddy kinetic energy by conversion of potential energy and its transfer to the mean zonal motion by the long waves in the westerlies; chapter 12 deals with the basic energetics of the general circulation with particular emphasis on the role of heat sources and sinks, and chapter 13 the relationship between internal and potential energy in the process of generation of kinetic energy on the synoptic scale. Chapter 14 (the longest in the book) is devoted mainly to an extensive development of the concept of available potential energy. Chapter 15 discusses the results of computations of the energy partitions and transformations between the mean zonal flow and the eddies using atmospheric data. The last three chapters are concerned with theoretical results on the transfer of wave energy, the role of ageostrophic motion in the energetics of the general circulation and the consistent formulation of simplified mathematical models of the atmosphere satisfying particular conservation laws.

Little comment is required concerning part 1 of this book and the first three chapters of part 2. The presentation is clear and the whole very carefully written. The chapters on free and forced small-scale convection are particularly well presented and include good examples of observed data illustrating the theory. Chapters 11 to 15, however, devoted to the energetics of the general circulation and comprising nearly half the book, although containing a wealth of mathematical theory, again very carefully and rigorously presented, somehow seem to lack a well defined sense of direction. The mathematical development is in places long and even tedious with little motivation (or apology!) supplied to the reader—for example in the section devoted to the first and second time derivatives of the total potential energy. Some improvement could be achieved by a more informative choice of chapter and section headings — what, for instance, is the reader supposed to infer regarding the distinction between the material in chapter 11 entitled "The Energetics of Large-scale Eddies" from that in chapter 14 entitled "The Energetics of Quasi-static Motion Systems"? The latter title seems in any case to be a self-contradiction! But these are essentially criticisms of presentation; the material in these chapters, like that in the rest of the book, conforms to the highest standards of scientific rigour.

The last three chapters are each more or less self-contained and relatively easy to follow.

There is little doubt that this scholarly treatise will rapidly become established as a standard reference book for research workers in dynamical meteorology. It probes to a more fundamental level than any other existing work into the consequences of applying the principle of energy conservation to the atmosphere. In doing so it leaves the reader with a deeper appreciation of how the atmosphere works. Perhaps, at the same time, it will leave some wondering whether there is very much more left to be discovered by attempting to refine still further mathematical methods for applying this particular principle to atmospheric dynamics without, at least, first thoroughly examining the consequences of applying the theory as it now stands.

R. P. PEARCE

Inside stars

Physics of Stellar Interiors. By Thomas L. Swihart. (Astronomy and Astrophysics Series.) Pp. x+119. (Pachart: Tucson, 1972.)

THE author of this book is well known for a textbook published five years ago in which he managed to give a remarkably concise and equally clear introduction into the fundamental concepts of astrophysics and stellar astronomy for the benefit of physics students. Three years later he wrote a short text which deals in less than eighty pages with the basic physics of stellar atmospheres and succeeds well in giving to the more advanced physics student a good general idea of the various aspects of this all important subject.

The success of these earlier books and their proven usefulness in the teaching

Language maths

Formal Languages. By Arto Salomaa. (ACM Monograph Series.) Pp. xiii+322. (Academic (Harcourt Brace Jovanovich): New York and London, May 1973.) $19.

THIS excellent book is unique in its scope, detail and elegance. It covers almost the whole of formal language theory up to early 1972 in more or less detail, always with great clarity of style. The emphasis throughout is on pure mathematical theory—there is almost no discussion of applications. The author assumes a considerable mathematical stamina in his readers, and although the rigour of the text is considerably alleviated by many examples and side remarks, I would not recommend it as a first introduction to the field for most undergraduates.

The book is divided into three parts. The first (about a third of the book) is a very complete account of the classical theory of the basic hierarchy. The emphasis throughout is on the characterisation of language families by different means and the effects of various operations upon language families. Concepts of recursion theory are introduced informally at first but, in chapter 3, Smallyan's "rudimentary-predicate" characterisation is introduced as a descriptive device for type O languages. There is considerable new material, notably workspace theory. This section would form an excellent nucleus text for a short graduate course in formal language theory.

The second part—about half the book—is its real *raison d'être* for the professional reader. It amounts to a connected self-contained survey of all major and several minor developments in modern formal language theory. Topics covered—AFLs, controlled grammars of various kinds; Lindenmayer systems (very interesting); a very full discussion of context-free languages, including power series analysis, LR(k) and LL(k) grammars and ambiguity; and finally a sort of linguistic dustbin containing transformational, categorical, indexed, scattered context and probabilistic grammars. Some of these are treated rather briefly, but references to other fuller discussions are given. (Brief bibliographical and historical remarks follow each chapter.) The scope of this section, the detail and rigour of the proofs, and the pervading sense of mathematical elegance, make these chapters a unique reference source for the field. The author gives many fascinating side remarks and illustrative examples.

The third part seems like an appendage. It consists of a fairly standard account of decidability and a nice introduction to complexity theory. The former would have been better placed nearer the beginning of the book: the latter should either have been omitted altogether, or should have been expanded to include (for example) a discussion of polynomial-time results for nondeterministic recognisers. At present it is an uneasy compromise.

The author has tried to give a complete account of a large subject. One can question whether it was sensible to make the attempt at this time, when the field is expanding so rapidly. Perhaps the publishers could leave a few blank pages in the next edition so that readers could jot down new results as they appear.

The book is technically well produced. I could find no significant misprints.

PATRICK J. HAYES

Brain damage

The Working Brain: An Introduction to Neuropsychology. By A. R. Luria. Translated by Basil Haigh. Pp. 398. (Allen Lane: London; Penguin: Harmondsworth, April 1973.) £2.50; Penguin £1.25.

ONE of the main problems with physiological psychology or neuropsychology is the crudity of its techniques relative to the complexity and subtlety exhibited by its subject matter, the brain. In this book one of the world's most eminent neuropsychologists surveys his vast work on the psychological effects of what is perhaps the crudest technique, brain damage. His purpose is to substantiate the claim that the resulting dysfunctions can tell one much about the functional structure underlying the cognitive and perceptual properties of the human mind and the mechanisms by which they are manifest in overt behaviour.

In the opening part of the book, Luria discusses lesion methodology and rejects the simple 'localisation of function' procedure in which the dysfunction is described as the loss of a certain ability; a process to mediate that ability is specified, and finally this process is localised in the particular brain area damaged. It is clear that in a complex mechanism such as the brain there is not necessarily any simple mapping between the domains of functional and anatomical organisation. The outcome of brain damage, defined by anatomical criteria, could well be behavioural disturbance which does not reflect in any meaningful way the structure of the processes mediating normal functioning. Luria's answer to this problem is that a "detailed psychological analysis of the structure of the disturbance and the elucidation of the immediate causes of the collapse of the functional system" is necessary. But here is the paradox. If an adequate psychological theory of a given ability is a necessary prerequisite of a "detailed qualification of the symptom", what additional information about the psychological organisation can the study of brain damage give us? The question is whether the rest of the book provides an adequate answer.

Luria divides the brain into three principal functional units: the reticular activating system and the limbic system for regulating the arousal of the cortex and controlling emotional and motivational processes, the primary and secondary projection areas and nonspecific areas of the posterior cortex for processing and storage of sensory information, and finally the motor and premotor areas and frontal lobes for programming, regulation, and verification of activity. In the second part, the author summarises a large body of evidence, mainly from his own or associated sources, on the effects of brain damage in various cortical areas. This material is then recapitulated in the third part and organised in terms of psychological processes rather than anatomical criteria.

Although a summary of Luria's extensive work must be of value, the relatively naive reader has difficulty in evaluating the adequacy with which the dysfunctions are presented. Most of the descriptions are supported solely by clinical evidence and rarely are control data given for comparison. This difficulty is compounded by the appalling and often incomprehensible presentation of the figures; graphs have unlabelled axes and examples have unexplained legends. The theoretical versus descriptive status of the terms in which a syndrome is presented is often ambiguous, and I get the impression that Luria, in spite of his caution against 'the localisation of function' procedure, often reifies deficits. Luria's theoretical position is eclectic and he draws on many sources in specifying the psychological processes he thinks underlie the capacities of the mind. But the general theoretical mechanisms are presented in such an unspecified form that it is very difficult to decide whether brain damage really tells us anything new about psychological processes. A conclusion, which could find supportive evidence in this book, is that psychological theory is still too primitive to allow the proper use of the patterns of behavioural breakdown induced by brain damage.

ANTHONY DICKINSON

New book?

Quantum Statistical Properties of Radiation. By W. H. Louisell. Pp. xiv+528. (Wiley: New York and London, July 1973.) £14.

THIS book is really a second edition of the author's 1964 volume *Radiation and Noise in Quantum Electronics* published by McGraw-Hill. Why he has changed his publisher and title I cannot

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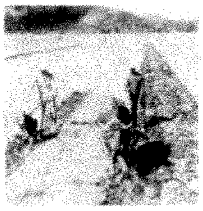

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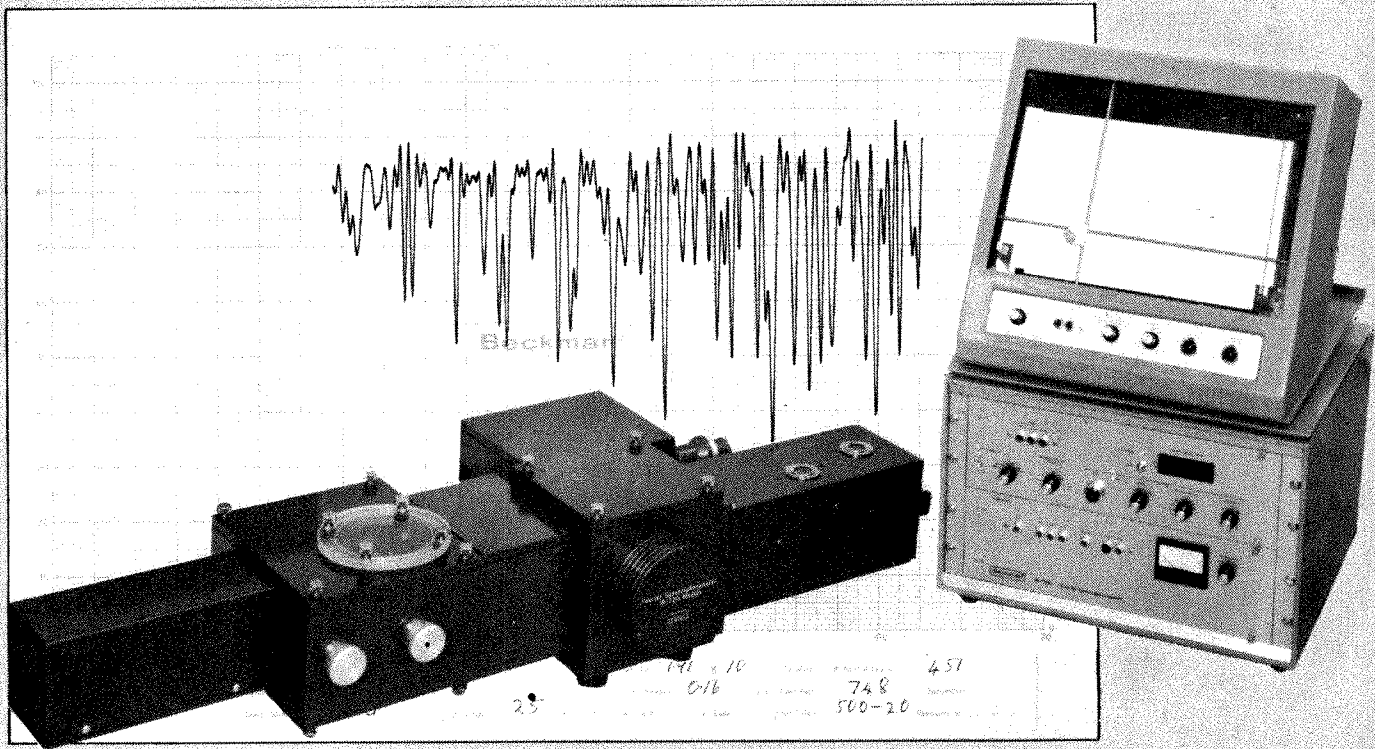

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nature

Volume 247 February 8, 1974

University research in danger

POSTGRADUATE education comes in for some very rough treatment from the House of Commons Expenditure Committee in its recent report (96-I, HMSO; £0.34). If the committee had its way the numbers of those going straight from undergraduate education to any form of postgraduate work would be cut. 'Post-experience' students, however, would be encouraged both to take vocational courses and to do research for a higher degree. Maintenance for postgraduates would include a repayable loan element but tuition fees would not be required of British students. Overseas students, on the other hand, would be expected to pay the full cost of tuition (about £1,500) except insofar as reciprocal arrangements and overseas aid schemes could be devised.

The brief given to the Education and Arts Sub-Committee of the Expenditure Committee was to examine the "financing and administration of postgraduate education". It is important to remember this brief, because the committee clearly exceeded it in venturing into discussions on the character of postgraduate education. And, having strayed from its brief, it inevitably obtained incomplete evidence.

The nine members of parliament on the committee, none apparently with any firsthand experience of the sort of postgraduate study under discussion, heard evidence from most of the bodies concerned with universities and polytechnics and from many industrialists. It also travelled to seven other countries, where its witnesses were mostly concerned with the financing of students.

The committee, unsurprisingly, discovered that there are divided opinions about the value of higher education and doctoral research. It heard that industry, in general, cares little for the PhD and prefers to recruit at the graduate level. International Computers Limited said their needs for postgraduates were "negligible". The Confederation of British Industry (CBI) described a "widespread industrial view that the system is producing a body of specialists in science and technology, the relevance and originality of whose research is often questionable". This ill-considered remark, out of place in the committee's terms of reference, epitomises the woeful relationship between industry and most university departments. Industry views PhDs as poor material, PhDs view industry as a poor place for their skills.

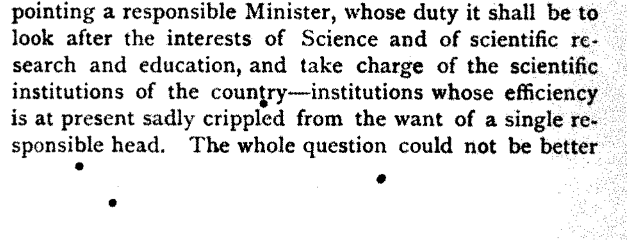
Having unearthed this unhappy relationship, it would have been well to call for a much fuller enquiry. The committee should have recognised the problem and faced it head on with a much greater attempt to understand the world of postgraduate learning. This was not to be; there is no indication that the committee appreciated why people do postgraduate research, what is unattractive in industry for the PhD and *vice versa*. Some of its foreign travel could have included attempts to find out why industrialists elsewhere do not share this contempt for the PhD.

The half-informed committee concluded that the the solution to such problems as there obviously are is to let industry decide. Rather than question industry's miserable record in attracting and employing PhDs, the committee was content to go along with it in ripping the present system apart. "We see [postgraduate education] as specialised training for mature students who normally will have shown talent and determination well above average both in the academic and professional worlds". So the pre-experience student is to be discouraged, except from taking vocational courses.

If pre-experience study were restricted, would post-experience students grow in numbers? No doubt a demand could be created for vocational courses of up to a year. But would industry really release employees for three years to do a PhD? It does not specifically say that it would, indeed one major employer says privately that it would not. So the PhD would be a narrower qualification, dominated by those in the academic field and even less in tune with societal needs.

The government would do well to reject this poorly reasoned report, even though times are hard and relevance is the watchword. The structure of research training should not be pulled apart just because British industry and PhDs have not come to terms. What is good for the CBI may be appalling for the world of learning.

100 years ago



WE are glad to see that the *Times* has at last opened its pages to the question of the propriety of appointing a responsible Minister, whose duty it shall be to look after the interests of Science and of scientific research and education, and take charge of the scientific institutions of the country—institutions whose efficiency is at present sadly crippled from the want of a single responsible head. The whole question could not be better

From *Nature*, **9**, 247, February 12, 1874.

Bringing science and technology to the world's forgotten people

David Spurgeon, Director of Publications at the International Development Research Centre in Ottawa, discusses a unique approach, funded by the Canadian government, to applying science and technology to the problems of the Third World.

ONE of the most conspicuous ways in which science and technology have not lived up to their promise is in bettering the lives of the world's poorest people. The majority of these people (about 70%) live in the rural areas of some 100 developing nations, where the applications of science and technology have rarely penetrated. Large numbers of others leave the countryside seeking better conditions, but then find themselves residents of wretched squatter settlements surrounding metropolitan centres. Very little effort has been made even to try to apply science and technology directly to the problems of these, the world's forgotten people.

Robert S. McNamara, president of the World Bank, outlined the problem at the bank's annual meeting in Kenya last September:

"Despite a decade of unprecedented increase in the gross national product of the developing countries, the poorest segments of their population have received relatively little benefit," he said. "Nearly 800 million individuals—40% out of a total of 2,000 million—survive on incomes estimated (in US purchasing power) at 30 cents per day in conditions of malnutrition, illiteracy, and squalor. They are suffering poverty in the absolute sense."

In 1970, the Parliament of Canada passed an Act setting up a public corporation designed specifically to help such people. Called the International Development Research Centre (IDRC), its purpose is "to initiate, encourage, support and conduct research into the problems of the developing regions of the world and into the means for applying and adapting scientific, technical and other knowledge to the economic and social advancement of those regions. . . ." The brainchild of former Canadian Prime Minister Lester Pearson and UN Environmental Agency Executive Director Maurice Strong, the centre became the first agency of its kind.

Unique approach

What is unique in its approach is that, unlike most aid agencies, it provides funds for the scientists of developing countries to carry out their own research projects in areas of their own choosing, corresponding to their own national priorities. At the same time, it attempts to build up research capability in these countries so that the indigenous problem-solving process can be perpetuated, and to promote regional communication in science and technology.

Last July the centre published a booklet called *The First 100 Projects*, which showed that in the 30 months from the inaugural board meeting in October 1970, to the meeting held in Bogota, Colombia, in March 1973, its board of governors had approved 100 projects costing $13.4 million in some 60 countries. For the most part, these projects were concentrated on improving the well-being of rural people, and the agricultural projects were concentrated in the world's poorest areas, the semi-arid tropics.

The IDRC's funds are appropriated by the Parliament of Canada, but the Centre is governed by an international board whose members come from ten other countries as well, six of them developing countries. A small professional staff in Canada and at several regional offices around the world encourage applicants to formulate projects for approval by the governors, who also set the centre's policy. For administrative purposes, the projects are placed in four divisions: Agriculture, Food and Nutrition Sciences; Information Sciences; Population and Health Sciences; and Social Sciences and Human Resources.

An agricultural project that illustrates one approach the centre is taking to rural research can be found among small farms in the Andean foothills around Caqueza, east of Bogota, Colombia. The IDRC supplies funds to the Instituto Columbiano Agropecuario not just to increase crop and animal yields, but to improve the marketing system, provide farmers with easier access to agricultural credit and fertilizers, provide training for graduate students, professional staff and farmers and their wives, and supply a low-cost health service. The farmer himself is incorporated into the program: research is carried out on his land as well as on experimental farms.

Health and social change

The health of rural people is a concern of the Population and Health Sciences Division, which is seeking new ways of providing health services appropriate to the needs and resources of developing regions. New approaches to family planning are also being undertaken. But equally important are studies of the dynamics of population change: how and why populations grow or diminish in specific areas, what the influences are on fertility rates, and the social factors governing child-bearing decisions and decisions of people to migrate.

Understanding of the forces that produce change, the process by which change occurs, and its social, economic, political and cultural consequences are the goals of social science projects. One of the first of these was a study of slums and squatter areas in eight cities, from Lima through Lagos to Seoul, the reasons and the ways they grew. Another is a study of the role of hawkers and vendors, who play an important role in the marketing and distribution of agricultural produce in Asian cities. Still others are concerned with formation of technology policy.

Obviously, then, rural people are not the sole concern of the centre's programs. Owners of small industries are the clients, in another example—an industrial extension service set up in Southeast Asia which is designed to strengthen national technical information services already existing there. Specialized information centres (for example, on cassava, geotechnical engineering and irrigation) are also supported.

While it is too early to gauge the effects of all this activity, IDRC personnel have been gratified by the response they have received so far from those they work with in developing countries. Nevertheless, difficulties do arise, and one of the chief ones is a direct result of the centre's insistence upon scientists from the developing countries learning through their own mistakes. It is also a result of the heritage of an agrarian tradition, often intermixed with an overlay of colonialist attitudes from the past, and of present-day paternalistic practices of some more conventional aid programs.

Learning by doing

The difficulty lies in a lack of experience in research management in many developing countries. After so many years of having donor countries run their projects for them, developing country scientists, faced with the opportunity of doing their own thing, often do not know how to go about it.

Even formulating a proposal may be difficult, because someone else has always done it for them in the past, and because they think they must frame it in terms that will please the IDRC representative. Centre representatives sometimes have to say: "Look, it's *your* project. How do *you* want to handle it?"

Such difficulties mean a loss of research time. One project lagged for months while an African country looked for ways of buying equipment in foreign currency without controverting its own currency rules, which forbade sequestering of foreign funds. Yet IDRC officials remain convinced that developing countries should, as far as possible, be left to work out such problems for themselves, because this is an essential part of the learning process. "I'd rather let a project lag a year behind than go in and tell them how to do it", says one director. "I am willing to sacrifice some quality of research for the experience gained in the process of doing research", says another.

Such an attitude is not always appreciated by those in developing countries. If their scientists have worked with other donor agencies, they may feel a lot of time could be saved if routine matters like supplying equipment could be handled for them. And their politicians sometimes want technical experts from developed countries sent in because they want to see results in a hurry. The IDRC only provides experts when local trained persons are impossible to find in a recipient country, and when the country asks for it—and even then they work as advisers with the project staff, rather than telling the local scientists what to do.

A distinction can be made between the management of a development project as a whole and the management of scientific research. Both seem to pose problems in developing countries. IDRC directors claim that, when a development project fails, it is rarely for lack of will, resources, or knowledge, but for lack of management capability. The difficulty lies in putting all the pieces of the puzzle together.

In research management, the problem is not only one of identifying problems and agreeing on goals, but also one of logistics. One laboratory in southeast Asia, for example, was found to have 50 years' supply of glass tubing in one size but none whatever in any other size.

National coordination

On a national level, a general lack of coordination of development activity exists, although Malaysia, for example, has achieved success by adopting a sort of "war-room" approach, observing the progress and interaction of the whole attack on development from the point of view of a single operation, as was done in war-time with military activities. The Philippines have adopted a similar systems engineering approach.

In outlining these problems of developing countries, I do not wish to suggest that the so-called developed nations have all the answers. Far from it. We can hardly expect developing nations to agree on national goals and coordination of research when we have not been able to do so ourselves. And that is surely the case, as a study of recent science policy literature will amply confirm.

Nevertheless, the IDRC is trying to do something about such problems, and indeed already has had some success. Centre funds are being used increasingly to bring scientists from developing countries together to talk about their mutual problems, and a workshop is being sponsored to encourage education in research management and to assist in identification of problems.

One meeting funded by the centre brought together nine senior scientists from as many different countries, no two of whom had ever met before. A result of this meeting was the establishment of a course in post-harvest technology of rice for middle-level managers in rice processing plants. A number of similar courses were later set up elsewhere. Funding was really all the centre provided in this case: the scientists themselves identified their own problems and ran the meeting.

In another instance, the centre encouraged the agricultural credit bank in an Asian country to provide revolving credit for local farmers by the simple expedient of setting an example by first doing so itself. Credit had never before been available to these farmers, but when they and the bank saw what it could do for them, the bank followed suit.

A question of balance

Not all types of projects are equally popular in the Third World. The benefits of an agricultural or a health delivery scheme are fairly obvious, and many countries make proposals in these areas. But the value of, say, a computerized data base or agricultural research information system is harder to envision for traditional societies, despite their enormous potential. Apart from anything else, developing countries are far more likely to have agricultural or medical experts of their own than information scientists.

In a statement to the board of governors' annual meeting last March, IDRC President David Hopper identified eleven policy issues in the centre's novel approach to foreign aid. All involved a question of balance. For example: How much should the centre follow the priorities of developing nations, and how much should it exercise its own judgment as to the "proper" priorities for development? How much should it emphasize the training aspect of research at the expense of first-class results? What should be the balance between support for research and direct support for post-secondary or postgraduate training, particularly in countries where training is vitally needed? What should be the balance between problem-oriented or applied research (so far emphasized by the centre), and phenomenon-oriented or basic research (now obviously necessary in some areas before further progress can be made)?

Perhaps the greatest test of the impact of the IDRC's approach to the field of foreign aid will be the influence it exerts on other international agencies to imitate its style of operation. There is evidence that these agencies are watching the experiment with more than usual interest.

international news

Defence, energy and Keynes shape US Science budget

Colin Norman, Washington

MEMORIES of the golden days of the early 1960s, when research budgets in the United States were lush with money, were carefully revived this week by President Nixon's budget proposals for the 1975 fiscal year. A massive increase in funding for the federal government's research and development programmes has been proposed—if it is all spent, it will be the largest single increase in funding for science and technology in more than a decade.

Unfortunately, however, there is absolutely no guarantee that the money will be spent. And, in any case, a careful look at the budget figures suggests that the overall prospect for research and development is not as rosy as it appears at first sight. In fact, some programmes will actually be cut back, and the increases being proposed for many others are insufficient to keep pace with inflation—particularly if it continues at its present galloping rate.

The budget—a collection of figures, facts and promises, richly laced with rhetoric—has been sent to Congress where it will be chewed over by the appropriations committees. The figures should be treated with extreme caution, for they are far from being money in the bank. For one thing, the spending proposals can, in theory, be drastically altered by Congress. And, for another, even if Congress agrees to the proposals, the Administration may later refuse to spend the money. It was, after all, only two years ago that President Nixon proposed large increases in spending on research and development, but ended up by impounding several hundred million dollars in order to stave off inflation. Nevertheless, the importance of the budget is that it provides a good indication of the Administration's overall policies for science and technology.

The sharp increase proposed for federal research and development owes its existence to three chief factors: a staggering increase in the budget for defence-related science and technology; an equally spectacular jump in the proposed outlays on energy research and development; and the Administration's partial espousal of the teachings of John Maynard Keynes—in an effort to head off a general recession, an expansionist budget has been proposed and science and technology will get a share of the largesse.

The overall strategy for research and development outlined in the budget is to increase expenditures on programmes likely to help solve pressing domestic problems—such as energy research and development—and to pump up the defence budget. But, on the other hand, expenditures in most other areas will be held back.

Part of the story is contained in Table 1. The overall increase in obligations for science and technology is a massive $1,626 million—up from $17,930 million this year to $19,556 million in 1975. (Obligations represent budgetary commitments, which may involve expenditures spread over several years.) But defence-related research and development alone is set to increase by more than $1,000 million, and energy research and development is earmarked for an increase of $816 million—from $999 million this year to $1,815 million next. Simple addition shows that the combined increase for those two areas is greater than the total increase for all federally sponsored research and development. Some programmes are thus clearly being cut to accommodate them.

One area to suffer is space technology—NASA's space budget will decrease by almost $200 million, chiefly because of the end of the Apollo and Skylab programmes. The research and development

TABLE 1 Conduct of Research and Development
(in millions of dollars)

Department or agency	Obligations			Expenditures		
	1973 actual	1974 estimate	1975 estimate	1973 actual	1974 estimate	1975 estimate
Defense—Military functions	8,382	8,573	9,581	8,417	8,676	9,201
National Aeronautics and Space Administration	3,085	3,309	3,122	3,271	3,104	3,173
Health, Education and Welfare	1,844	2,332	2,228	1,791	2,191	2,354
Atomic Energy Commission	1,361	1,429	1,702	1,361	1,429	1,709
National Science Foundation	480	530	654	428	460	538
Transportation	311	358	396	312	342	364
Agriculture	371	393	412	349	389	416
Interior	254	287	510	235	282	428
Commerce	191	210	266	179	192	233
Environmental Protection Agency	181	174	336	145	180	264
Veterans Administration	74	85	94	75	85	94
Housing and Urban Development	58	65	70	48	58	67
Justice	35	52	56	24	46	56
All other	176	132	128	150	151	125
Total, conduct of research and development[1]	16,802	17,930	19,556	16,784	17,585	19,016
Total, conduct of research	6,478	7,287	7,607	6,428	6,971	7,483
Total, conduct of development	10,324	10,643	11,950	10,356	10,613	11,352

[1] Details may not add to totals due to rounding.

Table 2 Research and Development in Colleges and Universities (in millions of dollars)

Department or agency	Expenditures		
	1973 actual	1974 estimate	1975 estimate
Health, Education and Welfare	937	1,159	1,232
National Science Foundation	329	339	427
Defense—Military functions	218	204	203
National Aeronautics and Space Administration	127	97	91
Atomic Energy Commission	83	84	94
Agriculture	88	95	99
All other	106	128	136
Total	1,888	2,107	2,283

activities of the Department of Health, Education and Welfare also appear to have suffered a cutback, but the picture there is clouded by the fact that the 1974 figure is swollen because it included funds held over from 1973. Other areas, with a few notable exceptions, are being held constant.

Asked last week whether he believes that the money proposed for research and development outside the energy and defence areas is sufficient, Dr H. Guyford Stever, Science Adviser and Director of the National Science Foundation, said that it represents a correct ordering of priorities for this year. In a press briefing, called to explain the budget for research and development, Stever claimed that civilian science and technology will enjoy an increase of nearly $1,000 million under the Administration's budget proposals. The proportion of the federal research and development budget devoted to defence and space research will thus drop from 68% to a mere 65%, continuing the trend established during the past few years (see Fig. 1).

By far the greatest increases will come at the development end of the research and development spectrum—a fact which is consistent with the emphasis on defence and energy research and development. Spending on research is set to increase by $320 million, from $7,287 million to $7,607 million. At just over 4%, that is clearly insufficient to keep pace with inflation. As for research and development in colleges and universities, expenditures are set to go up by about 7% under the budget proposals, with much of the increase coming from the National Science Foundation.

Overall, the prospects for research and development held out by the budget look bright. But what are the chances that Congress will go along with the proposals? As far as energy is concerned, it can safely be assumed that in an election year, and with the energy crisis constantly in the headlines, Congress will be unwilling to cut budget requests aimed at easing the energy shortages—in fact, the reverse is probably true. In other areas, however, the budgetary prospects are more difficult to predict.

The way in which the budget proposals would affect various branches of science, and individual government agencies, is discussed in more detail below.

Energy Research and development. Reporters attending the press briefing given by Dr Stever were handed three pieces of paper containing details of the budget proposals for energy research and development. The details differed substantially from one paper to another, a fact which not only added to the general obfuscation, but which also bears witness to the extreme haste with which the energy research and development proposals were thrown together. The final amounts were, in fact, settled only a few days before the budget was sent to Congress, and appeared as an appendix to the main budget proposals.

The chief outlines of the energy research and development proposals were revealed by President Nixon in January (see *Nature*, **247**, 247; 1974), and although some of the numbers have changed slightly since then, the overall thrust has not been altered at all. In short, an increase of 81% has been proposed for energy research and development, with coal research singled out for a particularly large increase. Nuclear fission is set to carry off about 40% of the $1,815 million that has been budgeted for all energy research, and solar and geothermal energy are earmarked for large increases.

The objective of the energy research and development programme is to exploit domestic energy resources as rapidly as possible, in order to offset imports of oil from the Middle East. The project has been given the name Project Independence by President Nixon, who has vaguely defined its goal as creating a capacity for energy self sufficiency in the United States by 1980. Last week, however, John Sawhill, Deputy Director of the Federal Energy Office, outlined just as vague, but rather more modest (and realistic) an aim for Project Independence. "By 1980", he said, "we want to demonstrate to the rest of the world that we are well on the way to energy self-sufficiency".

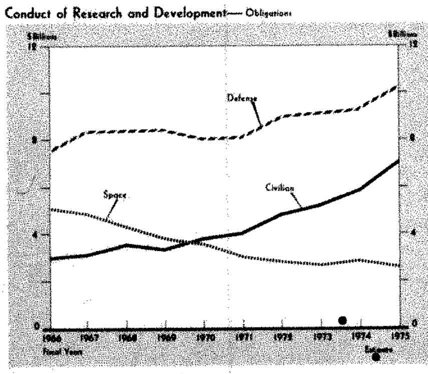


Fig. 1 Conduct of research and development-obligations.

National Defence. Although the United States is no longer fighting a war, and although the number of men in the armed services has been reduced, the budget request for the Department of Defense is a record $87,700 million, an increase of more than $7,000 million over this year's defence budget. Within that total, the amount proposed for research and development is set to increase from $8,573 million to $9,581 million. In addition, the Atomic Energy Commission is proposing to spend $631 million on defence-related activities, an increase of $24 million over this year's outlays.

The budget documents are characteristically vague about how this massive amount of cash will be spent, and Dr Stever, whose brief as Science Adviser does not include the defence half of the research and development budget, could only offer the information that about $80 million falls into the category of research, while the rest is labelled development. The Department of Defense is proposing to spend about $203 million of its research and development budget in the universities—about the same amount as in the past two years.

Biomedical research. Once again, the politically sensitive areas of cancer research and research into the causes and prevention of cardiovascular and pulmonary diseases have been favoured by large budget increases, at least partly at the expense of other areas of biomedical research. The Administration's budget request includes $600 million for the National Cancer Institute (NCI), which represents an increase of $73 million over this year's budget, and $309 million for the National Heart and Lung Institute (NHLI)—an increase of $22 million. The combined budgets of all the other institutes at the National Institutes of Health would, however, remain static. In other words, if inflation is taken into account, all the institutes except the National Cancer Institute and the National Heart and Lung Institute will suffer budget cuts if the Administration's spending proposals are agreed to by Congress.

The picture is, however, slightly distorted by the fact that the Office of Management and Budget impounded large amounts of money earmarked for NIH in 1973, but released it in 1974, thus swelling the 1974 totals. A slightly clearer picture can be gleaned from the proposed expenditure levels in 1975 (as opposed to obligations), which are set to increase for every institute. Nevertheless, the 1975 budgets for all the institutes, again with the exception of the NCI and NHLI, are lower than the budgets in 1973.

Last year, when the Administration's spending proposals for biomedical research were announced, they provoked an outcry in the biomedical research community over the plan to scrap the NIH training and fellowship programmes. Under strong pressure from Congress, Caspar Weinberger, Secretary of Health, Education and Welfare, announced in June last year a revamped NIH fellowship programme, to support postdoctoral trainees in priority areas of biomedical research. The new programme was given $27.5 million this year —enough to support 1,825 fellowships—and it is set to get a further $27.5 million in 1975 under the Administration's budget plan. Those amounts are, however, much less than Congress has been pushing for, and thus the fight over biomedical training seems far from finished.

Another major change being proposed by the Administration in 1975 is the phasing out of the General Research Support Program, a programme funded from NIH's Division of Research Resources, which provides general building support to institutions in proportion to the amount of biomedical research funds they receive from NIH. The programme is being phased out, according to the Department of Health, Education and Welfare, because "the institutions receiving this general support are now among the strongest research institutions in the world".

National Science Foundation. Like most of the agencies on the federal research landscape, the National Science Foundation has been selected to share in the large increases in funding proposed for energy research and development. Its total budget is set to increase from $646.4 million this year to $788.2 million next year, a jump of $141.8 million. Of that increase, however, $133.4 million is for energy related programmes, thus the rest of the NSF budget is what Dr Stever calls "tight".

Nevertheless, the NSF's programmes in support of basic research fare particularly well. The scientific research project support programmes, for example, are earmarked for an increase of some $72.4 million (of which $53.6 million is for energy related programmes) and will reach a total of $363.7 million if the Administration's budget proposals are accepted by Congress. The budgets for the national research centres are also set to increase, from $42.5 million to $52.5 million, with 13 million included in the budget of the National Radio Astronomy Observatory for construction of the Very Large Array telescope system.

NSF's programme of Research Applied to National Needs (RANN), an applied science programme which has suffered mixed fortunes at the hands of Congress, is set for a large increase in funds, from $73.8 million to $148.9 million—all of the increase being soaked up by energy research and development. Within that total, an extra $36.8 million will be heaped into solar energy research, and $16.6 million is earmarked for research on geothermal energy.

To accommodate some of the increases in energy research funding, some programmes have, however, suffered cuts. Most prominent casualty is the Experimental Research and Development Incentive Programme, an effort begun just two years ago which was aimed at testing methods for encouraging innovation in technology-based industry. The programme is being slashed by $11.3 million, and will move, according to Dr Stever, "into an evaluation mode". That seems to be a euphamism for the programme's termination, for it is left with only about $1 million in 1975.

Space research. Although the National Aeronautics and Space Administration is again hard pressed for money, space science has fared relatively well. The Venus Pioneer programme has been given the green light, and a powerful new infrared telescope is to be built on Mauna Kea in Hawaii.

NASA officials have managed to survive this year's budgetary battles without losing any major programmes—the first time for several years. Nevertheless, the agency's budget is still well below the $3,500 million level which Dr James Fletcher, NASA Administrator, said last year is needed for a balanced programme. Chief casualty of the financial stringency is the space shuttle, whose development schedule has slipped by about six months—the first manned orbital flight is now expected to take place in mid-1979 instead of towards the end of 1978. Fletcher said last week that he has obtained assurance from OMB, however, that here will be no further slippage in shuttle development.

The Administration's budget includes $27 million for the Venus Pioneer programme. The idea is to fly two missions to Venus in 1978, one of which will send four entry probes into the Venusian atmosphere, and the other will place a spacecraft in orbit around the planet. Both missions will be launched by Atlas-Centaur rockets, which is a change from original plans to use Delta launch vehicles. NASA found that the savings from removing some weight constraints outweighed the extra costs of the launch vehicles.

As for the infrared telescope, it will eventually cost some $6 million. It will be a 3 m telescope, located 14,000 feet above sea level, and its chief use initially will be to provide data on Jupiter and Saturn necessary for planning the Mariner Jupiter-Saturn flyby mission which is due to be launched in 1976.

Other notable features in the NASA budget are that the go-head has been given for SEASAT, a satellite which will measure the physical characteristics of the oceans; launch is set for 1978. The ERTS-B satellite has been rescheduled and will now be launched early in 1975—a year earlier than planned.

High Energy Physics. Once again, the 200 BeV accelerator at National Accelerator Laboratory at Batavia, Illinois, is being given a large increase in operating funds, largely at the expense of the other particle accelerators. The 200 BeV machine is due to get $36 million in 1975, an increase of about $7.6 million. Meanwhile the Alternating Gradient Synchrotron is set for a modest increase of $240,000 and the Stanford Linear Accelerator will get an extra $400,000, the Zero Gradient Synchrotron is being cut by $600,000, and the Bevatron will suffer a cut from $4.2 million to $1.7 million. According to a spokesman for the Atomic Energy Commission, however, the drastically reduced funding for the Bevatron does not indicate that the AEC is closing it down—it will, in fact pick up some extra funding from other parts of the AEC budget.

Bright ideas

John Hall

A PLEA for long term plans to use solar energy rather than entering a "Faustian bargain" to employ a nuclear technology was made by Professor D. Bryce-Smith (Department of Organic Chemistry, University of Reading) at the inaugural meeting of the United Kingdom section of the International Solar Energy Society in London recently. Professor Bryce-Smith urged a change in the climate (of opinion) so that short term economic considerations which dictated a nuclear strategy should not prevent the investment of money in a long term energy solution based on the power of the Sun. It was an appeal calculated to meet with immediate approval, since it was delivered to a meeting which filled the lecture theatre of the Royal Institution to overflowing with devotees of the solar economy.

What the faithful witnessed, apart from this brief obloquy against a nuclear power programme, was a quartet of lectures on solar possibilities, nicely illustrated with a slide show, and a clutch of demonstrations managed by Professor Sir George Porter in the best theatrical traditions of the institution. The only spontaneous round of applause during the entire meeting was accorded to a model railway engine which obligingly described a circuit whenever a bright light was directed on to its battery of photovoltaic cells. Alas, a further setpiece, meant to illustrate how light energy might be stored by a reversible process of chemical change, failed to function on account of technicality, and though belief in the theory can scarcely have been suspended, in many minds there was a distinct sense of pique at having been cheated of a spectacle. (Why is an elementary demonstration of a footling principle received like a virtuoso performance of conjuring by an assembly specifically equipped to see the simplicity of the feat? That is show-business.)

Gadgetry apart, Sir George made the point that although photochemical energy conversion might be a going concern in the field of space engineering, it is distinctly unpractical on economic grounds for generating power on *terra firma*. Apparently, the cost of making a 1 kw plant, using pure silicon crystals, is something in the region of £1,600. Polycrystals make the proposition cheaper, and metallurgical silicon is capable of offering a rate of £2 per kw of output. But given this cost, and the facts that the most efficient cell of this kind only converts 14% of the solar energy falling on it, and that this is difficult to store, a good deal of work needs to be done before photochemical energy conversion can make a sizeable contribution to the economy.

Professor J. K. Page (Department of Building Sciences, University of Sheffield) also made the point that the central research issue in solar energy is the question of storage. Speaking about the architectural requirements of successful solar usage, Professor Page pointed out that when we really want energy, in winter, the Sun does not offer all that much; and in summer we do not want all that is available.

He also, however, indicated that any reservations one might harbour about the unreliability of British summers is not in itself an argument against our benefiting from solar radiation, since direct sunlight is not essential to energy conversion; diffuse energy is available throughout the year and could be used in various ways. But, Professor Page suggested, perhaps the first priority is to conserve rather than harness the energy actually falling on the Earth. (In Australia in one year the amount of energy arriving from the Sun is equivalent to the total of the planet's reserves of fossil fuels, so they say.) The need, then, is to build houses which let in the heat when it is around and hang on to it as long as possible.

In the same area of bending the mind on comparisons of solar and conventional energy sources, Dr D. O. Hall (King's College, London) contended that the amount of biologically useful solar energy falling on the Earth in three days is equivalent, yes, to the total of the planet's proven energy reserves. Concentrating on photobiological energy conversion, Dr Hall calculated that if the sunlight falling on the United States in one year could be converted at 10% efficiency, only 1.5% of the land area would have been sufficient to provide the country's total energy requirements for 1970; and meanwhile, back in Australia, only about 0.03% of the land mass could have supplied the nation's needs.

Naturally, an obstacle to the realisation of this economic miracle is the harvesting and burning of the energy-rich products of photosynthesis. It would be a big job. Among priorities to improve the utilisation of solar energy by biological means, Dr Hall suggested the fermentation and pyrolysis of plant material to produce liquid fuels and gases; genetic engineering to produce plants which are more efficient as converters by photosynthesis, or plants which might be made to produce fatty acids or proteins; and the direct production of hydrogen by means of a photosynthetic system using water as the electron donor.

The ready production of cheap hydrogen (Dr Hill has not been the first to observe) would herald a new age of energy usage. Hydrogen obtained by photobiological means would use an inexaustible supply of energy (solar) and an inexhaustible supply of reagent (water) to produce a non-polluting energy. What price the new millenim?

Nader embarrasses the US AEC

Colin Norman, Washington

CRITICS of nuclear power in the United States have been engaged in the past two weeks in a brutal public battle with officials of the US Atomic Energy Commission over the safety of American light water reactors. The battle took place in the committee room of the Joint Committee on Atomic Energy, whose members were supposed to be holding the ring but ended up weighing in heavily on the side of the AEC. The charges and countercharges traded during the hearings are by now familiar stuff, but at least one attack grabbed headlines and put the AEC publicly on the defensive.

Ralph Nader, scourge of the automobile industry and consumer advocate *par excellence*, launched a blistering attack on the joint committee itself because "its zeal for promoting nuclear power has so overshadowed its responsibility to assure public safety that it has put at risk not only this generation, but a hundred generations to come". Nader also released an internal AEC task force report on the safety of nuclear power plants which, he said, shows that AEC officials have been "misrepresenting the facts" when they have assured the public of the safety of nuclear reactors.

AEC officials responded with some embarrassment to the disclosure of the task force report, suggesting that Nader had an early draft and that he was drawing false conclusions from the material it contained. Their response was to polish the report up and get it into the public domain as quickly as possible. They hope to publish a final version of the report sometime this week.

Nevertheless, Nader clearly scored points by unveiling the report in this manner. The task force, Nader said, "does not believe that there is the required confidence level that the probability for (a major) accident is one in a million or less per reactor year". Just a few days before Nader made his statement, Dr Dixy Lee Ray, the Chairman of the AEC, had indeed put forward the one-in-a-million suggestion as the basis for her contention that nuclear power plants are safer than most other forms of power generators.

The task force, Nader said, "assembled the most disturbing evidence of safety problems at US nuclear power reactors and documented the superficiality and incompetence of AEC licensing of nuclear power plants". Quoting directly from the report, Nader said that safety problems are "besieging" American nuclear power plants, and the level of safety of such power plants is "an unanswered question".

Most of the detailed examples of safety problems cited by Nader involve engineering defects which came to light after the plants were built. For example, he quoted a finding of the task force that five plants built by General Electric Company had, among other defects, broken hangar bolts, no lock nuts and improper bolts, and the deficiencies "could negate operability of the ECCS (the emergency core cooling system, which is supposed to flood the reactor core with water if the primary coolant is lost in an accident)".

Nader ended up by suggesting that the joint committe should dissolve itself because of its poor track record. But that suggestion did not go down too well with the members of that august body, and a verbal battle broke out. In particular, Mike McCormack, who was a nuclear chemist before becoming a politician, said that Nader's statements "revealed very profound ignorance of the whole subject".

Meanwhile, in Britain, the former General Manager of the US Atomic Energy Commission, Professor C. I. Wilson, listed the hazards of American light water reactors, in order of importance. First and foremost, he said in an interview with the *Times*, are doubts about the inherent safety of the light water pressure vessel containing the nuclear core, which could melt into "a radioactive puddle" in the event of a failure. Reactors of this type, he said, were "most unattractive animals to have as neighbors". And, if they really have to be built, he would rather see them 500 feet underground, in a sound geological formation.

An adviser on energy to the United States government, and a faculty member of the Massachusetts Institute of Technology, Professor Wilson went on to voice concern about nuclear reactors in general. After pressure vessel failure, the second most alarming prospect in his book is the production of large quantities of highly toxic material. The technology for handling the plutonium generated in breeder reactors is in its infancy, Professor Wilson said. Plutonium is the most toxic material known: AEC standards allow only half of one millionth of a gram to be absorbed by a person in a lifetime, but reactors are producing as much as 250 kg of plutonium each.

He is also anxious about the safety of future generations who are being left with a legacy of stored radioactive fission products. Although, he added, he has a degree of confidence that long term handling of the waste can be arranged safely.

correspondence

Energy strategy

Sir,—I am sorry that travel has kept me from responding earlier to your provocative leader 'A Broader Debate on Energy' (*Nature*, **246**, 179; 1973).

In kindly discussing my monograph, 'World Energy Strategies: Facts, Issues, and Options', you attempt what I did not dare—a brief summary of an extremely complex argument—and thereby, I fear, distort its thrust and substance.

I do not "see coal as the short and medium term solution to the energy problem", but as part of a diverse strategy stressing conservation and "unconventional" sources. Much better ways to mine, burn, and convert coal can help us to bridge to a sustainable energy economy, but are not a panacea.

You suggest that the paper ignores the social costs of coal. Careful reading does not support your view. Full weight is given to these high costs, which can be reduced but not eliminated. My "naive reference to a . . . deep mine which 'is said to use every kind of . . . precaution yet is still profitable' " is not an attempt to "let (coal) off the social hook", but a criticism of many operators for refusing, on economic grounds, to enforce precautions already known.

You refer to my "biased point of view . . . prepared to give coal the benefit of every doubt, nuclear energy the benefit of none". In fact, when all I knew about fission was what its proponents told me, I welcomed fission as a 'clean' alternative to coal. I am now prepared to give fission technologists full marks for zeal and cleverness, partial (and, in the USA, often undeserved) marks for diligence, and zero marks for recognising crucial gaps between intention and performance.

Many scientists far more distinguished than I are coming to appreciate the impact of human fallibility on a technology in which "no acts of God can be permitted" (Alfvén). Pugwash/Aulanko is one sign of a profound shift of opinion now underway. Another: last autumn, of 20-odd senior energy experts whom I saw in Washington, all were worried by fission and frightened by fast breeders; two-thirds wanted to go promptly to a fission-free economy. A year earlier, nearly all had been enthusiastic fission advocates.

A summary of recent convergence amongst sophisticated energy strategists cannot shrink from heterodoxy. It must attempt a thoughtful analysis of safeguards, safety, and waste disposal as they might be practised in the real world, not merely as they are designed in blueprints. References to Frisch's familiar paper are no substitute for the careful study that these issues deserve.

I could not agree more with your call for broad, holistic, up-to-date public and professional debate on Britain's long-term energy future. I am sure that many will join me in offering our help if you, Sir, are prepared to use your influence in practical initiatives to this end.

Yours faithfully,

Amory B. Lovins

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Bomb disposal

Sir,—Mr Davidson's suggestion of using liquid nitrogen for bomb disposal purposes (*Nature*, **246**, 548; 1973) is a technique invented by the Royal Engineers thirty years ago. The Germans had developed their 'Y' fuse which used mercury switches as an anti-handling device. The counter-tactic developed was to freeze the mercury in the fuse and then remove and dispose of the device. Liquid nitrogen, liquid oxygen, and a mixture of solid CO_2 and methanol were all used as refrigerants. The technique was completely successful. Plus ça change. . . .

Yours faithfully,

P. R. W. Webb

Office of Science and Technology,
Department of Industry,
Trade and Commerce
Ottawa

news and views

X-ray sources and Occam's razor

When two or more theories are available to explain the same observable phenomenon, it is usually a good bet to accept the simplest theory as the basis for further investigation. This powerful rule of thumb has many applications in astronomy, and 'works', for example, if used to assess the relative merits of theories of planetary motions. Straightforward elliptical orbits are simpler and easier to work with than complex interactions of cycles and epicycles, although admittedly the second model can be made to give the right answers. So can this philosophy be applied to the question of black holes, which is currently one of the most contentious issues in astronomy? Clearly, it can; the question which must be resolved is whether it is simpler to explain observations of objects such as Cyg X-1 in terms of black holes, or by invoking more conventional astronomical phenomena.

There is no doubt that the rapidly varying X-ray sources which burst upon the astronomical scene with the advent of the Uhuru satellite must be very compact objects. The only plausible energy source for the X-ray production mechanism is the release of gravitational energy as matter accretes onto a small, dense object—that is, a white dwarf, a neutron star or a black hole. Furthermore, the lengths of the periodicities found in some of these sources suggest, from even the most naive considerations, that the X-ray flux observed is being modulated by rotation or pulsation of just such compact objects. Perhaps it is natural that theoreticians should look for explanations involving the more extreme of these alternatives, since it is only by studying events occurring in such extreme conditions that theories can be pushed beyond their present limits of known reliability; however, there has recently been a tendency for models of these X-ray sources to move back one step towards the less exotic. In the case of Her X-1 and Cen X-3, it seems that white dwarfs, rather than neutron stars, are sufficient to explain the observations; in the case of Cyg X-1, a plausible model involving a neutron star rather than a black hole is put forward on page 351 of this issue of *Nature* by Fabian, Pringle and Whelan.

As far as Cen X-3 is concerned, the first suggestion that the periodic variations found could be explained in terms of white dwarf pulsations came very soon after the discovery of these periodicities (Gribbin, *Nature, phys. Sci.*, **233**, 18–19; 1971). But although that model "has the merit of providing a ready explanation for the fluctuations in the periods . . . which could be related to changes in the property of the star caused by the impingement of . . . gas streams", in 1971 attention remained focussed on neutron star models, and few people wanted to know about mundane explanations in terms of white dwarfs. Recently, however, DeGregoria and Woltjer have taken this idea a step further (*Nature phys. Sci.*, **246**, 108–109; 1973). They have investigated both Her X-1, which has a period of roughly 1.24 s that varies in a "rather complex" way, and Cen X-3, which shows a roughly 4.8 s period. It seems that certain models of pulsating white dwarfs which failed to account for the pulsar phenomenon can explain these X-ray variables. The mass inferred for Her X-1 is 1.3 $M_\odot$, and DeGregoria and Woltjer point out that the 1.24 s period of the source corresponds to the first harmonic vibration of a pure helium white dwarf model with a mass of 1.2 $M_\odot$, according to Faulkner and Gribbin (*Nature*, **218**, 734–736; 1968); similarly, the 4.8 s variation of Cen X-3 can be explained in terms of a white dwarf of mass 0.6 $M_\odot$.

One powerful argument in favour of these models is that "no plausible evolutionary scheme exists to explain the presence of a neutron star in . . . HZ Her" but "many binaries with white dwarf companions do exist" and there is no reason why mass transfer in these systems should not lead to X-ray production. "Models involving pulsating white dwarfs should not be prematurely rejected", say DeGregoria and Woltjer.

The model for Cyg X-1 put forward by Fabian *et al.* is a little more exotic than these white dwarf models—but less exotic, perhaps, than models involving black holes. There seems little doubt that if Cyg X-1 is a binary then the X-ray emission is associated with a compact object so massive that it must be a black hole. But is Cyg X-1 a binary? About a third of observed binaries are actually triple systems, and if Cyg X-1 is one of these then the mass problem can be removed. If the secondary is itself made up of two stars, a 1 $M_\odot$ neutron star orbiting a 10 $M_\odot$ B1V star, the observed modulation of the light curve of Cyg X-1 can be explained.

The conclusion is that "as yet there is no need to invoke the existence of black holes to explain the properties of X-ray sources". That may come as a disappointment to those relativists still searching for a spectacular proof that general relativity holds even in extreme conditions; but the mere suggestion that black holes might have been discovered has produced a wealth of theoretical ideas encouraging rapid development of a better understanding of high energy astrophysical processes, acting as a catalyst in a way reminiscent of how the steady state theory encouraged the development of cosmology in the 1950s and 1960s.

It may be that when the impossible has been eliminated whatever remains, however improbable, must be the truth. But alternatives to the black hole explanation of Cyg X-1 are not, it seems, impossible after all. J. G.

Cysteines at the interface

One of the most intriguing aspects of haemoglobin chemistry has been waiting more than a century for an explanation in structural terms. Since its discovery by Körber in 1866, the striking resistance of human foetal oxyhaemoglobin (Hb-F) to alkaline denaturation, in comparison to the adult protein (Hb-A), has been exploited by clinical chemists for the analysis of mixtures of Hb-F and Hb-A It is practicable to work at a *p*H, around 12.6, where the times for half-denaturation differ by two orders of magnitude (about 1000 s for Hb-F compared with about 10 s for Hb-A), and much ingenuity has been shown in devising convenient routine procedures and in extending the sensitivity and reliability of the methods to low proportions of Hb-F.

It has often been suggested that this difference in stability of Hb-F and Hb-A arises from the ease by which the tetramer molecule dissociates into dimers and monomers. Since the work of Itano and colleagues in 1967 and of others, the higher resistance of Hb-F to dissociation at both low and high *p*H has been well established. The present knowledge of the tertiary and quaternary structure of haemoglobin, and of the differences in the amino-acid sequences of the polypeptide chains of a large number of haemoglobin species, has now been used by Perutz (see page 341 of this issue) to provide an explanation for the large variations in their rates of alkaline denaturation.

In accordance with earlier views that the rate-limiting step is likely to be the formation of monomeric subunits from the dimer, Perutz has looked at sequence differences located at the $\alpha_1\beta_1$ interface for evidence in support of his hypothesis that denaturation by alkali is activated by the ionization of buried, weakly acidic side chains. Comparing Hb-A with bovine haemoglobin, which is almost indefinitely stable at *p*H 12.7, Perutz concludes that the significant structural difference between these two haemoglobin species in the region of the $\alpha_1\beta_1$ contact is the presence of three buried weakly acidic groups—two cysteines and one tyrosine—which will ionize above *p*H 12 in Hb-A, and which are replaced by neutral or more weakly acidic groups in bovine haemoglobin. The ionisation and hydration of these groups perturbs the tertiary structure and therefore shifts the equilibrium towards the unfolded state and favours dissociation across the $\alpha_1\beta_1$ interface. Comparing Hb-A and Hb-F, the non-α or γ chains of the latter species provide one less cysteine and one less tyrosine in the same locations. These are two of the three changes, relative to Hb-A, found in bovine β chains. It is in fact possible to list several haemoglobin species in order of increasing resistance to alkaline denaturation, and to show that this order follows the progressive substitution of β-130 tyrosine, β-112 cysteine and, finally, α-104 cysteine by neutral or more weakly acidic residues.

Thus both qualitatively and semi-quantitatively, Perutz's suggestion that the ionisation of buried weakly acidic side chains determines the relative susceptibilities of different haemoglobin species to alkaline denaturation seems to be well founded. Certainly electrostatic effects involving exposed ionising groups are likely to be involved in the denaturation of monomers, but the ionisation of buried weakly acidic groups, notably cysteines, in the dimer interface region seems to be the crucial factor in promoting monomer formation at high *p*H and initiating the sequence of events leading to denaturation.

From a Correspondent

Herpes not so simplex

THE most recent discovery of Sabin and his colleague Tarro may open up at least two interesting avenues of research, both of which will be pursued at the University of Chicago, but each of which lead in an entirely different direction. Sabin announced early last year the finding, now published (Sabin and Tarro, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3225; 1973), that the serum of patients with certain types of tumour contains antibody to a substance produced by cells infected with herpes simplex virus (HSV). The discovery, which swells the growing body of evidence for the involvement of herpes viruses in human cancer, (see, for example, *Oncogenesis and Herpesvirus*, edit. by P. M. Biggs, G. de Thé and L. N. Payne; International Agency for Research on Cancer, 1972) is of immediate practical interest because the presence of antibody is associated with metastasis and may thus constitute a diagnostic tool for monitoring the progress of malignant disease. But its more far reaching implications depend on the possiblity that the antigen is specified by a fragment of the HSV genome expressed in the tumour cells. From the practical point of view, this would establish the rationale for developing the anti-viral vaccine which Sabin has envisaged as a prophylactic against human cancer. From the point of view of basic research, the antigen seems at last a palpable tool for probing the mechanism of carcinogenic transformation of human cells.

But although Sabin and Tarro refer to it as a nonvirion antigen of HSV, no definitive evidence yet exists that it is specified by viral DNA. The evidence linking the tumour antigen with HSV is strong but circumstantial: all tumour types which have so far produced positive serum reactions in complement fixation tests occupy sites which would be accessible to HSV in a typical infection of the mouth, by HSV type I, or the genitals, by HSV type II.

Lac repressor and operator sequenced

How does repressor select one out of several million nucleotide sequences and bind to it to prevent the expression of the operon genes? In order to understand repression one must understand this and the stereochemistry underlying it. Two signal advances to this end have been reported by Gilbert and Maxam and by Beyreuther, Adler, Geisler and Klemm in the current issue of the *Proceedings of the National Academy of Sciences* (**70**, 3576 and 3581; 1973). Briefly Gilbert and Maxam have sequenced the operator, a stretch of DNA which binds the repressor of the *lac* operon in *Escherichia coli;* Beyreuther *et al.* have sequenced the repressor itself.

Gilbert and Maxam made use of the fact that the purified repressor will bind to cellulose nitrate those fragments of sonicated DNA which carry the operator. After binding in this manner the washed fragments were then eluted with a synthetic inducer. The repressor was found to bind to these purified fragments with a half life of 15 min so a quick treatment with DNase could be used to digest away all sequences except those protected by the repressor. The result was a double stranded DNA with a T_m of 67° C in SSC and containing twenty-seven base pairs as revealed by a calibrated polyacrylamide gel.

The sequence of this DNA was ascertained by copying one or other strands using RNA polymerase and complementary oligonucleotides of different length of sequence to effect initiation at various points. Fingerprinting of the transcription products yielded the complete sequence.

A feature of some interest in the final result was an ACAATT sequence near the 3′ end of each strand (with a complementary sequence near both 5′ ends), which gave the operator an element of symmetry. The two regions giving this symmetry are separated by nine base pairs and four of these show the same pattern of symmetry; the two regions are about one helix turn apart and thus could be approached from one side by a repressor showing a two-fold symmetry.

The repressor itself turns out to consist of four identical subunits each of 347 residues. No similarities with the sequence of histones or the known part of the β galactosidase were detected. Exactly how the two bind together must await further investigation, but Gilbert and Maxam suggest possibilities like opening up the DNA to see the bases directly or feeling out the edges of the bases in the grooves.

From a Correspondent

Sabin obtains the antigen from massively infected cultures of rabbit cells, in which it makes a brief appearance 8 h after infection, or guinea pig cells, in which it is synthesized about 3 h after infection (*Proc. natn. Acad. Sci. U.S.A.*, **70,** 1032; 1973). The designation nonvirion rests on the persistence of reactivity in serum which has been absorbed with the structural components of HSV. The instability revealed by Sabin's tests implies either that it is rapidly degraded or that it is an aggregate of molecules the antigenicity of which is different from those of its components. It is reasonably clear that it arises as a consequence of HSV infection; but it is not at all clear that it does not represent a newly synthesised or modified preexisting host antigen. Similarly, the antigen which induces antibody in cancer patients may result, by chance, from similar but independent modification of human cell components.

The true identity of the antigen is the point which the laboratory of Roizman, at the University of Chicago, is well equipped to clear up. This group has been engaged for the past five years in a comprehensive analysis of the polypeptides specified by HSV and their synthesis in infected cells. They are also interested, though from a rather more academic point of view than Sabin's, in the involvement of HSV in the aetiology of cancer. At any rate, some of their resources have now been recruited to the pursuit of the putative nonvirion antigen, with the use of Sabin's sera to precipitate the polypeptide(s) involved. If they succeed, Sabin will have established the incrimination of HSV for all practical purposes; although, of course, the practical problems do not end with the identification of the causative agent.

The problems which would be encountered in an attempt to reproduce with HSV Sabin's achievement with poliomyelitis are legion. The potential dangers of a vaccine containing a virus which can remain latent in human cells for years and which, even when killed, can transform cells (Duff and Rapp, *Nature*, **233,** 48; 1971) need no emphasis. Even supposing it proved possible to isolate a mutant which neither remained latent nor transformed cells *in vitro*, on which to base a killed vaccine from which the viral DNA was removed, it seems likely that safety criteria for testing on human populations would be very hard to establish. Besides which, the vaccine might prove relatively ineffective in preventing infection, which can occur even in the presence of circulating antibody.

From Roizman's point of view, on the other hand, an established viral product expressed in tumour cells would provide the link he needs between two parallel lines of work in his laboratory. One concerns polypeptide synthesis and its regulation in infected cells, which involves separation of the viral polypeptides on high resolution gels at various stages of infection. This has so far revealed forty-seven viral polypeptides, between sixteen and twenty-six of which are non-structural and therefore possible candidates for Sabin's and Tarro's non-virion antigen. They can be further classified into five different groups according to their rates of synthesis at different stages of infection (Honess and Roizman, *J. Virol.*, **12,** 1347; 1973). The other line of work concerns the fragments of the HSV II genome detected by N. Frenkel, with Roizman, in the cells of tumours of the uterine cervix. Frenkel is following up the discovery with studies of DNA hybridisation of HSV fragments from different cervical tumours, in an attempt to isolate the sequence common to all of them. Sabin's and Tarro's nonvirion antigen could represent a product of the common genome fragment to which a specific function might possibly be assigned on the basis of the continuing investigation of the synthetic programme in HSV infected cells.

It would be a mistake to suppose that because the unstable nonvirion antigen is the only possible product of HSV so far detectable in tumour cells, it must necessarily be the functionally crucial product. But as the only one on hand, it is inevitably suspect as the transforming agent itself. In this case, according to his current hypothesis on the activities of HSV, Roizman would expect to find, not that the antigen would trigger mitosis, but that once mitosis had been initiated, it would keep the cell in the mitotic cycle. He has suggested that HSV remains latent only in differentiated cells which do not normally divide: on mitosis, the cell produces a factor which is necessary for the initiation of viral transcription. When the entire genome is present in the cell, expression results in the production of infectious virus and the destruction of the cell. But if, as in the case of tumour cells, only a portion of the genome is present, expression results in transformation of the cell.

To establish the role, if any, of the unstable antigen in such a mechanism, substantial advances in the fundamental understanding of HSV function would be necessary. Assigning regulatory functions to viral products will involve, among other things, the use of numerous mutant strains of HSV. Here, once again, Sabin and Roizman have an interest in common. Sabin needs HSV mutants from which to select one suitable for development as a vaccine. Roizman needs them to tie viral function to viral product. Perhaps coordinators of cancer research should give up urging pure and applied researchers to seek conceptual common ground in favour of getting them together on purely technical requirements.

From our Special Correspondent

Dark imaginings

from our Experimental Psychology Correspondent

ONE of the reasons for the current interest in visual imagery is the question why a picture is worth a thousand words. Certainly this issue is very much the core of Allan Paivio's influential book *Imagery and Verbal Processes* (1971) and is, I suspect, also behind the recent paper of Neisser and Kerr of Cornell University on spatial and mnemonic properties of visual images (*Cognit. Psychol.*, **5**, 138; 1973). In two experiments Neisser and Kerr succeed in giving further confirmation of one answer to the question: visual images do provide a powerful aid to memory. But they also invite a series of related conclusions about the nature of spatial thinking and of perception.

It is worth mentioning that psychological interest in visual imagery as a mnemonic device dates back at least to classical Greece. The classical method, which is described in Frances Yates's admirable book *The Art of Memory*, is based on the proposition that sequences of concepts, such as one might want to use in giving a speech, are best committed to memory by imagining oneself walking round a familiar building, and creating in the successive places that one passes, visual images of the things one wants to mention.

There were within the theory all sorts of instructions about how to create memorable images: they should be concrete, striking, with clearly defined visual properties such as being beautiful or grotesque. There were also rules about the best kind of building in which they were to be put. It should be thoroughly familiar, but not crowded with people. It should not be of an architecturally repetitive style, but it should have discriminable locations, such as niches or corners of rooms, which should not be dark or in shadow.

More recently, and now cast into the arguably more rigorous experimental paradigm, these rules of visual mnemonics have been both re-established and extended. Thus it has been shown that just as in the ancient art a well-known building created an associative structure, so too, if one wishes to remember an association between two words, say, dog and bicycle when learning a list of such word pairs, the most efficient strategy is to visualise a scene in which some interaction between the dog and the bicycle occurs: say, an image of a dog riding a bicycle, or being run over by one. This produces better recall of the second word bicycle when cued with the first, than does the creation of separate images of dog and bicycle or than an attempt simply to remember the words.

This, then, is the background of Neisser and Kerr's experiments. Subjects were given a series of twenty-four descriptive sentences, asked to form visual images of what was described in each, and to rate each image for vividness. For eight of the sentences the scene could be pictured directly, for example, "A harp on the top of the torch of the Statue of Liberty". For eight there was a connection between the two objects, but one that could not be captured in a single picture because of concealment; so the corresponding sentence would be "A harp inside the torch of the Statue of Liberty". For the remaining eight there was no direct connection, for example, "Out of one window I can see a harp and out of the other I can see the Statue of Liberty".

The second experiment of Neisser and Kerr provided some obvious controls for complexity and length of the sentences, and added a condition in which there was minimal luminous contrast between the two objects to be identified, for example, a piece of coal in a dark windowless cellar. The subjects were not warned in either experiment that they would have a recall test, but Neisser and Kerr found that when cued with the context given in each sentence (for example, the torch of the Statue of Liberty which for any one subject would occur in only one of their twenty-four sentences) subjects were able to recall the other object mentioned in the sentence equally well whether the image were pictorially possible, or when the object was concealed, or when there was low visual contrast between the object and its context. For disconnected images, however, recall was substantially worse, and there was furthermore no correlation between either time taken to form the image or its vividness when the sentences were first presented, and its recall subsequently.

Neisser and Kerr conclude that the mnemonic power of images depends upon their being representations of spatial relationships, not upon their possibilities for comprising a good 'picture' as such. The lack of decrement in the poor visual contrast images, for example, coal in a cellar, seems to be one point of contradiction with the rules of the ancient art of memory, which proposed that the loci in which images were to be placed must be well lit.

Just as interesting as the use of visualisation in memory are the implications that this kind of investigation has for perception and thinking. Neisser and Kerr do not fail to relate their findings, that visual images are three-dimensional representations of layout rather than two-dimensional pictures of scenes, to an understanding of visual perception. When one sees, one does not experience retinal pictures as such, but the spatial layout of the environment. What they might also have pointed out, but did not, is that human beings seem to possess specialised and powerful mechanisms for reasoning about spatial relationships. They form the basis of visual perception and Helmholtz referred to them as processes of "unconscious inference". In the Renaissance the ancient art of memory became transformed from a process of simply recording static images to a method that actually allowed the powerful mechanisms of spatial conceptualisation and inference to help one to think. Perhaps soon one may also look forward to some experimental exploration of this aspect of the uses of imagery.

Interaction between pineal and pituitary glands

from our Steroid Biochemistry Correspondent

THE pineal gland has been studied intensively in recent years and the view is growing that this gland can affect several endocrine functions in the body. It is known to inhibit reproductive functions in mammals. Although the mechanisms by which the pineal acts are not fully understood, it is thought that light, and possibly other factors such as cold, stress or other hormones, act on it to decrease the synthesis of melatonin and/or serotonin by inhibiting specific enzymes needed for the formation of these compounds. Production of melatonin in the pineal is stimulated by darkness and inhibited by light. Several questions about the synthesis, secretion and transportation of melatonin and its effect on different endocrine glands, however, remain unresolved. Do these compounds act peripherally at specific sites or is their main site of action within the brain affecting trophic hormone release? Are they secreted into both the blood stream and cerebrospinal fluid?

Further information relating to some of these questions has now been provided by Seibel and Schweisthal (*Acta endocr., Copnh.*, **74**, 434; 1973), who used the golden hamster to study the relation between the pineal and other endocrine glands. Although melatonin or serotonin have not yet been isolated from the pineal gland of the hamster, this animal has the advantage in studies of the effects of changes in environmental lighting and pinealectomy, that the changes in the gonads are much greater than in the albino rat.

The mean weights of testes and seminal vesicles of hamsters blinded by bilateral enucleation were significantly lower than those of control, pinealectomised or blinded and pinealectomised animals, but could be increased by melatonin, whereas after hemi-castration, pinealectomy with hemi-castration or with hemi-castration and blinding there was a significant increase in the mean weight of the testes. Mean testicular weight was not altered by bilateral enucleation combined with hemi-castration suggesting an interaction between the pineal antigonadotrophic substances and the pituitary gonadotrophins, but was reduced on treatment with melatonin and serotonin.

No significant changes in the weights of the adrenal or pituitary were found in any experimental conditions except in the group which underwent all three operative procedures and were also treated with melatonin. These results differ from those obtained in the rat where regression of the adrenals occurs after blinding. The increased uptake of iodine-131 observed in blinded animals could be reduced by melatonin treatment. After parabiotic union in males, whether one or both animals were blinded, there was a significant decrease in testicular weight indicating that the pineal antigonadotrophic substance was carried through the blood.

These investigations show an interaction between the secretions of the pineal and those of the pituitary. Melatonin is known to depress secretion of luteinizing hormone most probably by an effect on the hypothalamus. So does serotonin which will also depress secretion of follicle stimulating hormone and the relations between these two biogenic amines needs clarification.

Genetic manipulation

from a Correspondent

GENETIC manipulation by the biologist has, in the past few years, become an area of great controversy and increased research activity. This has led, in some cases, to workers trying to jump on the genetic engineering bandwagon when their results are only minimally applicable to the field.

Levy, Snyderman, Ladda and Lieberman (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 3125; 1973) attempt to show that a genetic deficiency can be temporarily repaired after the inoculation of Sendai virus fused cells *in vivo*. The recipient animals are mice which are deficient in the ability to produce the fifth component (C5) of complement. The inoculated cells are splenic macrophages fused to either kidney cells from a closely related mouse strain (C5 positive) or chicken erthyrocytes. Splenic macrophages are thought to be at least one of the cells involved in the production of complement whereas neither mouse kidney cells nor chicken erythrocytes are involved in C5 formation.

Sera taken from the inoculated animals were shown to contain antigenically-related mouse C5 as well as both haemolytic and biological complement activity. The C5 activity reached its peak at 3-6 d after inoculation and then declined reaching background levels after 21 d. The decline in C5 activity may be due to the production of anti-C5 antibody by the recipient animals. Possibly the simplest explanation is that the environment for the production of C5 is defective in the mouse splenic macrophages. This defective environment may be complemented by an non-defective cell, even a chicken erthyrocyte, to cause the production of mouse C5.

These results are no doubt interesting but do not seem to warrant the authors' speculation that their experiments may provide a prototype for genetic repair by hybrid cells in humans. Although the authors continually talk about hybrid cells no evidence is shown that the fused cells indeed contain hybrid cells capable of division. Most cell biologists would refer to them as a heterokaryon-containing population. In any case fused cells are not essential for the result. Similar results were also obtained by Levy *et al.* by inoculating unfused non-defective splenic macrophages. As with the fused cells the repair with these macrophages is also only temporary. Even if the real and major problem of preventing the rejection of transplanted foreign cells could be overcome, viable functional cells (hybrid or not) which are capable of populating the appropriate areas within the recipient animals would be needed. Finally one should take into account the possible dangers to the host in such experiments. Fusion of cells can not only induce dormant viruses but can also result in differences in growth control and antigenic character in the hybrid cells produced.

A report more applicable to genetic manipulation is presented by Cohen, Chang, Boyer and Helling (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 3240; 1973). Using the restriction enzyme *Eco* RI these workers have been able to construct new biologically-active plasmid DNA molecules which are capable of replication. *Eco* RI cleaves double stranded DNA at a unique site producing short, overlapping single stranded ends. The overlapping sequences are self complementary and will hydrogen bond to each other allowing the nicked molecules to be resealed by DNA ligase *in vitro* or *in vivo*. By these means fragments from the same or different sources can be joined together.

Cohen *et al.* utilized bacterial plasmids which contain a number of drug resistance markers. Transformation was performed with the *Eco* RI endonuclease cleavage products of a plasmid DNA containing twelve cleavage sites. By selecting for resistance to only some of the markers Cohen *et al.* were able to isolate a clone of bacterial cells which contained a plasmid with only three *Eco* RI fragments. By mixing the *Eco* RI fragments of two different plasmids and using similar procedures hybrid plasmids were obtained. In all cases it was shown that the new plasmids were most likely derived from the joining together of *Eco* RI fragments. By identification of the fragments present and of the drug sensitivity of the plasmid an assignment of drug resistance to particular fragments was then made.

Every combination of restriction enzyme fragment hybrids can probably not be isolated. In order to be able to isolate the hybrid molecule a functional system for replication and transcription must be present. Since fragmentation and subsequent hybrid molecule formation depends on DNA sequences and not on the origin of the DNA, this method may well be used as the authors point out for inserting and growing up DNA fragments from a variety of sources. If DNA from higher organisms can be tolerated in bacterial cells, one will no doubt soon see the isolation of many genes. This procedure is not necessarily restricted to the use of *Eco* RI as there are a number of other restriction enzymes which produce overlapping single stranded ends and cleave at other unique sequences in the DNA.

Indirect effects of pesticides

from a Correspondent

Ways in which pesticides may affect the host plant, besides controlling pests and diseases, were considered by the Pesticides Group, Society of Chemical Industry, on November 6 under the chairmanship of R. A. E. Galley. Damage to a crop can take many forms and in some the symptoms are obvious. Effects on yield, though more important, are less easy to detect. Several speakers stressed the difficulty in demonstrating statistical significance even for a 10% change. They found that attempts to relate yield to dose of pesticide often led to unexpected results.

Petroleum oil emulsions, used to control red spider mite in glasshouses, may reduce cucumber yields by about 10% when sprayed every 10 days throughout the 8-month crop. N. W. Hussey (Glasshouse Crops Research Institute) described the symptoms produced by various oil fractions on young tomato plants. Single sprays applied undiluted at forty times the normal quantity showed that even a low aromatic content can cause damage, especially with oils of low viscosity. Removal of the aromatic constituents by percolation through silica gel largely prevents damage to both cucumbers and tomatoes. Trials are continuing on cucumbers sprayed with dearomatised oils at normal rates through the season.

D. J. Butt (East Malling Research Station) discussed hazards to shoots, blossoms and fruits arising from fungicide sprays used on apple trees. Fungicides interact with cultivars, time of season and weather, and must be selected and timed so that disease control is achieved with minimal phytotoxicity. The flowering period is vulnerable and, although mildew control is necessary at this time, dinocap and especially binapacryl can reduce fruit set. On occasions benomyl has increased fruit set. Crop quality can be reduced, thus binapacryl applied after June may cause spotting. The most insidious type of damage is russeting, and fruit skins are sensitive to certain fungicides in May and June. The systemic fungicides available tend to induce russet as does dodine when used near 0° C. Some fungicides ameliorate russet, and captan and bis(dimethylthio-carbamoylthio)methylarsine have been successful as palliatives (*Ann. appl. Biol.*, **75**, 217; 1973).

Factors contributing to the phytotoxicity of cereal seed dressings were considered by K. A. Jeffs (Rothamsted Experimental Station). Wheat plants from seed treated with gamma-BHC are less damaged in peaty than in sandy soil, but chlorfenvinphos has caused damage in peaty soil. Adhesives to stick powder dressings to seeds may enhance phytotoxicity, as may liquid formulations. Experiments with gamma-BHC liquid dressing showed that only seeds treated near the embryo were damaged and amounts of insecticide translocated into the plant from treated seeds were variable and small.

F. Baranyovits (Plant Protection Ltd) illustrated the safety of two insecticides to plants. Pirimicarb is systemic when applied to soil, being absorbed by the roots and transported through the xylem. When high doses are applied it accumulates at the lower leaf margins of chrysanthemums causing necrosis. Its distribution has been confirmed by autoradiography with ^{14}C-pirimicarb-treated plants. Pirimicarb smokes are safe at the recommended rate but an eight-fold increase produced varied damage. Thus with carnations the growing tips were scorched, only the older leaves of cucumbers were damaged, cyclamen were unharmed, and primula petals unaffected whereas the leaves were damaged. An experimental pirimiphos-methyl formulation produced localised scorch on citrus fruit when spray droplets dried concentrating the insecticide. No damage occurred with a formulation that dried more uniformly.

Flies in mushrom houses are controlled by mixing insecticides with the casing layer or with the compost though yield reductions have been suspected. I. J. Wyatt (Glasshouse Crops Research Institute) described trials in which diazinon and chlorfenvinphos (from 10 to 2,000 mg kg^{-1} casing) reduced mushroom numbers in the early flushes and increased them in the later. When numbers were decreased, however, the mushrooms were larger, resulting in increased yields with low rates of insecticide. The compounds were more persistent in the compost and tended to reduce yields in all flushes. A simple computer-fitted model described the behaviour. G. A. Wheatley (National Vegetable Research Station) also described unexpected effects produced by insecticides in trials. Chlorfenvinphos gave good control of cabbage root fly damage on mini-cauliflowers but the yield was less than with diazinon, which gave poor control. On conventional cauliflowers, curd diameter increased progresively with increasing diazinon dose, but not with chlorfenvinphos or fonofos although they gave better protection against cabbage root fly larvae. Phorate and *S-tert*-butyl *OO*-diethyl phosphorodithioate controlled carrot fly and affected plant survival. Although total yield did not change much, the size of the carrots was affected and hence their suitability for specific markets. Different ways of using chlorfenvinphos altered sizes of Brussels sprouts with similar implications.

E. Griffiths (University College of Wales, Aberystwyth) reviewed the effects of fungicides on leaf rust and coffee berry disease affecting coffee in East Africa. One spray of Bordeaux mixture doubled yields even in the apparent absence of disease. It became an annual treatment known as 'tonic copper spraying', though other fungicides produced the same effect and both diseases can be aggravated. Without further control measures rust may later develop more quickly in the thickly foliated trees that result from 'tonic spraying'. Coffee berry disease infection can come from berries or the fungus living saprophytically in the bark of maturing shoots. Fresh spores from bark sprayed 14 months earlier are more infective than those from untreated trees. Do the fungicides affect the tree or other microorganisms?

Introducing a lively discussion, Q. A. Geering (Fisons Ltd, Agrochemical Division) stresed that many of the effects described had been obtained with higher doses of pesticides than used commercially.

Insulator layer formed in silicon

from our
Solid State Physics Correspondent

THE frequency of publication on ion implantation indicates that this technique may be catching on as a technological tool (see, for example, the recent book *Ion Implantation* by G. Dearnealey, J. H. Freeman, R. S. Nelson and J. Stephen (North-Holland, Amsterdam, 1973). In the research journals and at conferences, every week seems to bring a new idea for making use of ion implants in microelectronics. A beam of ions projected at kilovolt energies into the surface of an electronic material forms a burned layer of implant atoms, in a matrix of atoms of the host material. The properties of that layer are, of course, different from the original material. For semiconductors it has been realised for some time that by varying the ion used, its energy and the subsequent heat treatments, one can form either a layer which is more strongly conductive than the original semiconductor or a glassy layer which is still a semiconductor but much less conductive. It has only been clear since about 1970, however, that, by implanting a high concentration of a reactive ion, a layer of insulating compound can also be formed.

Dexter and Watelski of Texas Instruments, Dallas, and Picraux of Sandia Laboratories, Albuquerque, (*Appl. Phys. Lett.*, **23**, 455; 1973) have now reported a particularly useful application of that finding. They have used bombardment wafers of silicon with nitrogen ions to form a buried silicon nitride layer and then, by etching grooves, they have produced slabs or 'mesas' of silicon which are isolated from the body of the silicon wafer. 'Dielectrically isolated' devices can then be formed in these slabs—a useful form of device in the preparation of fast interference-free integrated circuits. The surprising and novel feature of the implantation method was that, even with the high ion dose used (more than 10^{17} ions cm^{-2}) the silicon left above the buried layer was free of defects after heat treatment at 1,200° C. Proton channeling showed a near-perfect lattice and the silicon would support the epitaxial growth of a similarly defect-free crystalline silicon film of thickness 0.2 μm. The important point about the film quality was its superiority in comparison with a competing process, namely the epitaxial growth of silicon films on sapphire. The latter process has been used to manufacture integrated circuits but suffers from some drawbacks such as inhomogeneity of impurities and defects in the silicon and expensiveness and difficulty in working the sapphire.

The buried 'nitride' films had a breakdown strength which approached that of pure silicon nitride, breaking down at a field of 5×10^5 V cm^{-1}, although, theoretically, there is not enough nitrogen present to form the compound Si_3N_4. It seems that it will be possible to form diffused transistors and diodes in this silicon layer of 0.2 μm and interconnect them into an integrated circuit. This method could prove cheaper, simpler and hence more reliable than the present methods for dielectric isolation which, with the exception of the silicon-on-sapphire technique, involve the difficult stage of lapping off the body of the silicon wafer after mesas, formed by etching, have been somehow secured in a solid dielectric matrix.

A warning against overoptimism concerning this method should, however, be inferred from the almost simultaneous publication of an article describing unexpected migration of defects beyond the range of implanted ions themselves. Clearly, high dose implantation and the required high temperature anneals which follow, place a large stress on the electronic materials involved which may lead to new, unexpected and frustrating forms of damage effect.

Superfluidity in thin helium films

from our
Condensed Matter Correspondent

THE superfluid content of very thin adsorbed helium films has been measured directly, using a quartz crystal as a microbalance, by Chester and Yang of the University of California, Los Angeles (*Phys. Rev. Lett.*, **31**, 1377; 1973).

Bulk liquid ^{4}He below T_λ, its superfluid transition temperature, behaves in many ways as though it were a mixture of two entirely different but completely interpenetrating fluids a so-called normal fluid component which, with finite viscosity and entropy, is much like any other liquid; and a superfluid component which, having zero viscosity and entropy, is responsible for many of the remarkable phenomena associated with liquid helium. The proportion of superfluid varies from unity at 0 K to zero at T_λ.

Much interest has been attached to the behaviour of helium in confined geometries and, in particular, to the properties of adsorbed helium films held on glass or metal surfaces by the Van der Waals force. It is well known that superflow occurs readily in the relatively thick (100 atomic layers) films which occur in equilibrium under the saturated vapour pressure. An intriguing question arises, however, as to just how thin a helium film can be made before its superfluidity disappears and this is one of the problems to which Chester and Yang have addressed themselves.

Their apparatus consisted of a quartz crystal, 0.07 mm thick and 6.3 mm in diameter, vibrating in its thickness-shear mode such that the oscillations of the surface were parallel to itself. In this situation the characteristic frequency of the crystal is very sensitive to the mass of any material deposited on its surface and the mass of an adsorbate can be determined accurately by measuring the resultant drop in frequency. In the case of a helium film, however, only the normal fluid component is able to follow the oscillations of the crystal. The superfluid component, because of its zero viscosity, is not coupled to transverse oscillations of the surface, remains motionless and therefore does not contribute at all to the loading effect of the helium film. The technique thus enabled direct measurements to be made of the mass of normal fluid in the film, under various conditions of temperature and film thickness.

The film thickness at any particular temperature T was controlled by adjusting the pressure P of the helium gas surrounding the crystal and with which the film was in equilibrium. The mass σ_1 which was loading unit area of the crystal at first increased with P, suddenly fell by a small factor and then increased more slowly as P continued to rise towards the saturated vapour pressure, P_0. Chester and Yang interpret these results as indicating that at very small values of P (very thin films) the film displays no superfluidity; they attribute the abrupt drop in σ to the onset of superfluidity with an associated decrease in the effective mass loading the crystal; and they point out that the subsequent slower rise with P of σ_0 is to be expected, assuming that only the additional normal fluid will load the crystal. This interpretation is consistent with their earlier paper (*Phys. Rev. Lett.*, **29,** 211; 1972) with Stephens of the California Institute of Technology in which they presented preliminary data and, in particular, reported that similar measurements for an adsorbed film of ^{3}He, which is not a superfluid at these temperatures, yielded a monotonic dependence of σ_1 on P, from zero right up to P_0, with no sign of the features attributed to superfluidity in the present case of the ^{4}He films.

To deduce the proportion of superfluid component from the experimental values of σ_1 it was, of course, necessary to know the total mass σ per unit area of the film for any given values of T and P. By replotting their values of σ_1 against $(P/P_0)^T$ as abscissa, the authors have succeeded in generating a universal curve relating σ to P and T, as required. Each experimental curve started out from the origin along the universal curve and then, at some particular value of $(P/P_0)^T$, depending on T, started to deviate because of the presence of superfluid. By measuring the magnitudes of these deviations they were able to determine the mass σ_s of superfluid component per unit area corresponding to any particular value of σ, for a variety of temperatures.

After making additional assumptions, consistent with a number of earlier experiments, that the first adsorbed layer is solid, and that the second and subsequent layers are liquid of the standard density, the authors conclude that superfluidity does not occur in a helium film until between two and three atomic layers of liquid, depending on the temperature, have been deposited.

X-ray 'star' in Cygnus Loop

RAPPAPORT *et al.* report that they have detected a central 'hot spot' in the Cygnus Loop. This source, detected at X-ray frequencies during a non-dimensional scan from an Aerobee rocket on March 30, 1973, may be the stellar remnant of the supernova explosion, *Astrophys. J. Lett.*, **186,** L115; 1973). If so, it represents only the third definite identification of such a stellar remnant.

In a sense, this discovery completes a set of such identifications, for the central star in the Crab Nebula was first found with optical telescopes, and the equivalent source in Vela was first detected at radio frequencies, as the Vela pulsar. Both these other two objects, of course, have X-ray counterparts.

The best position quoted for the Cygnus object is $\alpha(1950) = 20\text{h}\ 49\text{m}\ 40\text{s}$; $\delta(1950) = 30°43'.5$. But the error box only marginally overlaps with that of the 'hot spot' reported by Stevens and Garmire (*Astrophys. J. Lett.*, **180,** L19; 1973), which lies distinctly off centre in the Cygnus Loop. The available observations are fitted by a blackbody spectrum with temperature $1.9 \pm 0.6 \times 10^6$ K, and X-ray luminosity of 8×10^{34} erg, assuming a distance of 770 pc. It is interesting, as Rappaport *et al.* point out, that these parameters correspond to an object about 30 km across, in the right area for a neutron star. Also, there is some evidence of periodic variations of the source with a frequency of 16 Hz, again about right for a spinning neutron star.

Swings, roundabouts and Sco X-1

from a Correspondent

THAT there is a source mechanism which can give rise to periodic pulsed radiation in close, interacting binary stars is rapidly becoming an established observational fact. A wide range of apparently differing systems which exhibit a variety of pulsing behaviour are being found.

The regular periodic pulses observed in the X-ray systems Cen X-3 and Her X-1, superficially similar to the on-off, on-off radio pulses of pulsars, are now well known. The periods, of the order of a few seconds, are so short that it has become widely accepted that a highly compact neutron star must be involved (although while dwarf models cannot yet be ruled out—see *Nature*, this issue, page 333). The widely accepted explanation of these stable pulses is that they are produced by the infall of material, flowing from the normal stellar component, and accreting preferentially along magnetic field lines onto a neutron star companion. If the material is funnelled in this way onto the magnetic poles and the neutron star rotates with the required period of a few seconds, the observed X-ray pulses are, at least qualitatively, easy to explain.

In the case of the strongest compact X-ray source, Sco X-1, no such stable pulsing behaviour has been found, suggesting the absence of a strong magnetic field. But several claims have been made that weak periodicities of a transient nature can be observed, in particular in the optical region. These are the subject of a recent paper (Frohlich, *Mon. Not. R. astr. Soc.*, **165,** 313; 1973) which may help clarify the issue.

The optical counterpart of Sco X-1 is known to be a variable star exhibiting very rapid light fluctuations, and additional larger scale flaring on a timescale of a few hours. The apparently erratic optical fluctuations have been analysed by various investigators using power spectra and the so-called periodogram techniques in a search for evidence of underlying periodic behaviour, with somewhat discrepant results. Of a total until now of seven investigations, only two teams found evidence of periodic behaviour; one group reported a transient pulse with a period of 20 s in both the optical and X-ray region; the other group suggested the detection of pulses in the optical with periods of 117 and 182 s coexisting.

The existence of pulses in Sco X-1 has therefore been a matter of some controversy. But the latest observations of Frohlich cannot be lightly dismissed. On four successive days in June 1972, evidence was found for the presence of low amplitude optical pulses with a period of 121 s. This agrees quite well with at least one of the periods found by an earlier group (P. A. Peldman *et al.*, *Nature*, **225**, 1123; 1970). A further fact, which if confirmed might help in the interpretation of these pulses, was a correlation between the brightness of the source and the period observed. On one night the overall output declined by a factor two in half an hour. This was associated with a decrease in the pulse period from 121 s to 118 s. Although Frohlich does not speculate as to the origin of these weak, transient pulses or this apparent brightness period correlation, a whole range of questions and possibilities immediately come to mind.

The sort of behaviour reported in Sco X-1 is remarkably similar to that of another class of binary system, the so-called dwarf novae. These show similar weak transient pulses which change in their period as the brightness changes. The dwarf novae are distinguished as a class by their explosive outbursts, brightening suddenly by several orders of magnitude and then more slowly declining back to a quiescent state. The outbursts recur at irregular, but approximately monthly intervals. The origin of these outbursts and of the underlying transient pulses remains a question of debate. But we might hope that the observation of somewhat similar pulse behaviour in Sco X-1 may help to elucidate the phenomena occuring in both types of system.

Two main lines of approach to account for transient pulses have so far been suggested: either oscillations, similar in some ways to the oscillations of the well known Cepheid pulsating stars; or rotation, as in the pulsars and stable pulsing X-ray sources mentioned earlier. Thus the choice seems to lie between what might be called swings and roundabouts. Both have been advanced to explain the pulses in dwarf novae. Put simply the swings model accounts for the pulses as being due to oscillations of the envelope of the accreting compact component, the pulses being excited by the nova outbursts themselves which are ascribed to phases of unstable nuclear burning on the accreting white dwarf. The roundabout model ascribes the outbursts to the sudden dumping of matter onto the dwarf as its companion undergoes phases of unstable mass outflow. It is well known that as a consequence of its angular momentum this accreting material must spiral slowly in onto the white dwarf rather than falling straight onto the surface. It is suggested that it is just irregularities in this inward spiralling disk that gives rise to the pulse phenomenon.

It will be interesting to see how the roundabouts and swings fare in explaining in their rival fashions the behaviour of Sco X-1. Perhaps one shall then be able to get an answer to the truth or falsity of that old adage about gaining on swings and losing on roundabouts.

Radiation of life in the Precambrian

from our Palaeontology Correspondent

THE cause of the sudden explosive radiation of multicellular organisms near the beginning of the Cambrian period, after a long history of slow unicellular evolution, is widely regarded as the foremost unsolved problem in palaeontology. In recent years two rather different approaches have been adopted in an attempt to solve the problem: the first emphasised external physical controls such as the changing level of atmospheric oxygen, whereas the second concentrated on biological aspects, such as the advent of eukaryotic cell organisation, sexuality or the formation of preservable skeletons. Neither approach, however, has been generally accepted.

In an important review, Schopf *et al.* (*J. Paleont.*, **47**, 1; 1973) point out that the evidence of Precambrian fossils suggests that eukaryotic organisms arose more than 1,300 and sexuality at least 900 million years ago. The advent of sexuality should greatly have increased genetic variability and hence rates of evolutionary diversification, but the late Precambrian floras seem to be very impoverished in comparison with their living equivalents. The authors suggest that the long time gap, of 250–300 million years, between the origins of sexuality and multicellular body organisation might have been a consequence of the gradual nature of the trend from a haploid to a diploid-dominated life cycle. This explanation does not seem very convincing, because an important evolutionary innovation such as diploid cell division should have risen to dominance very quickly. Another explanation of the rapid radiation of metaphytes and metazoans has been put forward by Stanley (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1486; 1973), who applies a newly established 'cropping' principle to the problem.

Ecologists have acquired a substantial body of evidence that the addition of a trophic level to a given food web tends to promote increased diversity at the next lower trophic level. Thus the removal of the chief natural carnivore from a community can lead to a drastic reduction in herbivore diversity as one or a few superior competitors among the latter achieve dominance. According to Stanley, the Precambrian trend from simple autotrophic producer to producer–herbivore and producer–herbivore–carnivore communities can be viewed as a long-term natural cropping experiment comparable to those performed by ecologists.

Initially an all-producer Precambrian organic world of bacteria and blue-green algae was characterised by low diversity and low rates of speciation. The system was self-limiting insofar as the changes needed to produce unicellular heterotrophs and metazoans had to arise slowly because diversification was slow. When cell-eating heterotrophs first arose in the late Precambrian they may have fed on bacteria. The adaptive breakthrough to feeding on algae led to the addition of successive trophic levels to the ecosystem. In general, diversification at any trophic level tends to promote diversification of the subjacent level by cropping and diversification of the suprajacent level by providing more feeding options. Thus a self-propagating mutual feedback system can arise. Stanley sees the explosive evolution of multicellular organisms as an inevitable consequence of the advent of heterotrophy. Supporting evidence is sought from the record of ancient stromatolites, which are laminated sedimentary structures produced by blue-green algal mats that trap and bind sediments. Their rapid decline at the close of the Precambrian has been considered to be the result of destruction of algal mats by animal grazers and burrowers, so that subsequently blue-green algae have only flourished in restricted environments, hostile to other organisms, such as supratidal flats. This suggests that blue-green algae and other photosynthetic autotrophs saturated aquatic environments until very late in the Precambrian.

It is obviously too early to assess what chance Stanley's theory has of being more popular than its predecessors. It does seem, however, to have several definite strengths: it accounts for a wide variety of facts, it is basically simple and is purely biological, with no *ad hoc* invocation of external physical controls.

Mechanism of denaturation of haemoglobin by alkali

M. F. Perutz

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Denaturation by alkali is activated by the ionisation of buried, weakly acidic side chains. Species variations are largely accounted for by replacements of one buried tyrosine and two cysteines.

Two effects may contribute to the denaturation of a protein by acid or alkali. First, the free energy of a spherical polyelectrolyte is proportional to the square of its net surface charge, so that its stability decreases on either side of the isoelectric point as either its cationic or its anionic groups are being discharged. Second, titration with acid or alkali may ionise weak acids or bases that lie buried in the non-polar interior. On becoming ionised, these groups attract hydration shells which are misfits in the native structure and therefore shift the equilibrium towards the unfolded form.

Mammalian haemoglobins have molecular weights of 64,500 and consist of four haems and two pairs of polypeptide chains known as α and β. The α chains each contain 141 amino acid residues and the β chains 146. Their secondary structures are made up of seven or eight helical and six non-helical segments (Fig. 1). Their tertiary structures are held together by secondary valency forces, mainly hydrophobic interactions. Side chains carrying ionised groups or amides are excluded from the interior of the subunits, but cysteines, serines, threonines and tyrosines occur both inside and outside. The four subunits are arranged tetrahedrally around a two-fold symmetry axis. In oxyhaemoglobin, which is the form under discussion here, significant contacts are made only between unlike subunits and these are restricted by symmetry to two kinds called $\alpha_1\beta_1$ and $\alpha_1\beta_2$. In both these contacts hydrophobic interactions predominate[1,2]. Carbonmonoxy and cyanomethaemoglobin have the same structure as oxyhaemoglobin.

Human and Bovine Haemoglobin

X-ray analyses and amino acid sequence studies show that the molecular architecture of all mammalian haemoglobins must be closely similar, yet they vary greatly in their susceptibility to denaturation by alkali[3]. For instance, in 0.1 N NaOH at 20° C adult and foetal human CO-haemoglobin are denatured with half times of 20 s and 12 min respectively, while bovine haemoglobin is stable. It takes 0.5 N NaOH to denature it with a half time of 3 h[4]. All three haemoglobins dissociate from tetramers into dimers above *p*H 10.0 (refs 4, 5). This step is reversible and not associated with any denaturation[5]. It is due to splitting of the molecule at the $\alpha_1\beta_2$ contact[6] where the amino acid sequence is invariant, so that no significant species variations in the dissociation constant arise. The resulting dimer has the structure shown in Fig. 1 (ref. 7); its two subunits are held together at the $\alpha_1\beta_1$ contact which is made up of thirty-four residues or 110 atoms, mostly belonging to helices G and H and to the GH corner[1]. When the *p*H of human adult CO-haemoglobin is raised to 11.6, this dimer splits apart and denaturation sets in[4]. It has been suggested that the splitting is likely to be the rate-limiting step[8]. When considering its cause, it is useful to keep in mind the experience gained from the abnormal human haemoglobins which has shown that any amino acid replacement which opens the non-polar interior to water is likely to be a source of instability, and that this is true especially of buried non-compensated charges[9].

The amino acid replacements that distinguish the three proteins are known from sequence studies[10-14]. The adult human and bovine forms differ at fifteen positions in the α and eighteen in the β chains. One of these differences is a deletion of residue NA1 which lies in the internal cavity of the human tetramer, and twenty-six are found in positions which, in the tetramer, are either external or lie in the internal cavity. In the $\alpha\beta$ dimer this cavity is wide open, so that these latter positions also become external. Of the remaining six differences, three are due to exchanges of non-polar residues, two of them interior (EF2 and EF5α) and one lying in a surface crevice (A11β). Of the replacements listed so far, the only ones to be affected by raising the *p*H from 10 to 13 are the lysines and arginines whose cationic groups have *p*Ks of 10.5 and 12.5 respectively[15]. But the instability arising from their discharge is unlikely to have a decisive effect on the susceptibility to alkali denaturation, because there is no net difference between the number of basic groups in the two proteins and because human haemoglobin is known to remain native even after acetylation of 80% of its amino groups[16]. Of the remaining three positions, G11α and G14β are both occupied by cysteines in human adult, but by serine and valine re-

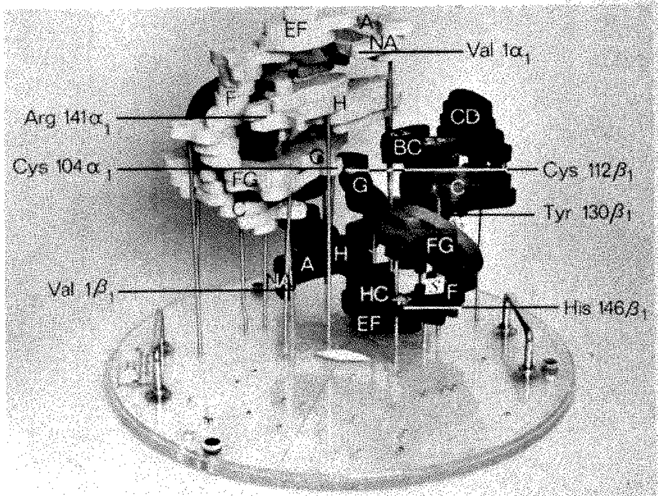

FIG. 1 Model of the $\alpha\beta$ dimer seen at low resolution. In going from the amino to the carboxyl end, helical segments are denoted A to H; non-helical segments at the amino end NA and at the carboxyl end HC. Non-helical segments between helical ones are denoted AB, BC and so on. Residues within each segment are numbered from the amino end (see Table 1). S marks cysteine 93β. The white sign on the base marks the position of the two-fold axis in the tetramer.

Table 1 Susceptibility to alkali denaturation of various haemoglobins as determined by Singer test*

Species	Cys G11 (104) α	Cys G14 (112) β	Tyr H8 (130) β	% undenatured haemoglobin after 1 min at pH 12.7	k†
Human adult[10–12]	+	+	+	~1	2.3×10^{-2}
Macaque (Rhesus)[26]	+	+	+	0 – 1.4	
Spider monkey[24]	+	+	+	0.5 – 0.7	
Capuchin monkey[23]	+	+	+	2.4 – 2.7	
Dog[20]	+	+	+	~3‡	
Rabbit[22,27]	+	–	+	?	2.8×10^{-4}
Slow loris[25]	+	–	+	53	
Human foetal[13]	+	–	–	80	
Brown lemur[22]§	+	–	–	67	
Bovine[14]	–	–	–	100	9.3×10^{-6}

* In the Singer test[36] 0.1 ml of a 10% solution of oxyhaemoglobin is added to 1.6 ml of N/12 NaOH (pH 12.7) at 20° C. After 1 min 3.4 ml of a solution made up to 800 ml 2 M $(NH_4)_2SO_4$ + 2 ml 10 N HCl are added and the mixture is filtered. The percentage of undenatured haemoglobin remaining in the filtrate is determined spectroscopically. The procedure was later modified by Beaven *et al.*,[37] using cyanomet- instead of oxyhaemoglobin but still measuring the percentage which remains undenatured after 1 min at pH 12.7 and 20° C.

† First order rate constant at pH 11.9 and 2° C (ref. 28).

‡ Inferred from the rate of denaturation published by Huehns *et al.*[19]

§ Based on peptide compositions only.

spectively in bovine, while H8β is occupied by tyrosine in human adult and phenylalanine in bovine. All three positions are buried, G11α and G14β in the $\alpha_1\beta_1$ contact and H8β between helices H and A. In water, the sulphydryl groups of cysteine have a microscopic pK of 9.1–9.5 and the phenolic groups of tyrosines one of 9.5–10.0 (ref. 15). These groups might have their pKs raised if they lie buried, but they would certainly become ionised above pH 12. The pK of the hydroxyl group of N-acetylserine amide in water, on the other hand, is 13.6 (ref. 17), which would be raised further in serine G11α in bovine haemoglobin, because it lies buried among the hydrophobic side chains. I conclude that the raising of the pH from 10.0 to 13.0 would lead to the ionisation of three buried and weakly acidic groups in human adult, but not in bovine, haemoglobin.

Human adult and foetal haemoglobins share the same α chains, but the β chains of the foetal form, usually referred to as γ, differ from the adult form in thirty-nine positions[13]. Twenty-nine of these are external in the $\alpha\beta$ dimer, seven involve replacements of one type of non-polar interior side chain by another, and one involves a replacement of an internal alanine in β by a threonine in γ. By the arguments set out above, none of these are likely to have a significant effect on the susceptibility to alkali denaturation. The remaining two replacements are of cysteine G14 in β by threonine in γ and of tyrosine H8 in β by tryptophan in γ. These are the same buried, weakly acidic groups which are replaced by neutral or more weakly acidic groups in the β chains of bovine haemoglobin. In summary, the differences in susceptibility to alkali denaturation are likely to arise because human adult haemoglobin has three buried side chains ionisable around pH 12.0, human foetal has only one, and bovine none (Table 1). The two buried cysteines lie at the $\alpha_1\beta_1$ contact; in the ionised form they would be hydrated and prevent the association of the subunits. Ionisation of the buried tyrosine would attract water into the non-polar interior of the β chain and tip the equilibrium towards the unfolded state.

Free γ chains associate to form tetramers and are more susceptible to alkali denaturation than native foetal haemoglobin[18], probably because the dissociation constant of the equilibrium $\gamma_4 \rightleftharpoons 4\gamma$ is larger than that of the equilibrium $\alpha\gamma \rightleftharpoons \alpha + \gamma$.

Other species

Information about the rate of alkali denaturation is available for several other species though not always in comparable form. The half times of the denaturation reaction of canine and adult human cyanomethaemoglobin at pH 12.7 and 20° C are similar: 20–25 s as compared to 11 s[19]. This is consistent with the presence in both species of Cys G11α, Cys G14β and Tyr H8β (ref. 20). Of the thirty odd primates studied[21], complete sequences are available for four and a partial one for another[22–26]. The haemoglobins of the rhesus, capuchin and spider monkeys (all adult) contain little or no alkali-resistant haemoglobin and Cys G11α, Cys G14β and Tyr H8β are all present. Oxyhaemoglobin of the brown lemur is denatured at pH 12.7 and 20° C at a rate similar to that of human foetal oxyhaemoglobin[21]; like the latter, it contains Cys G11α but not Cys G14β or Tyr H8β (ref. 22). Oxyhaemoglobin of the slow loris is denatured at a rate intermediate between that of

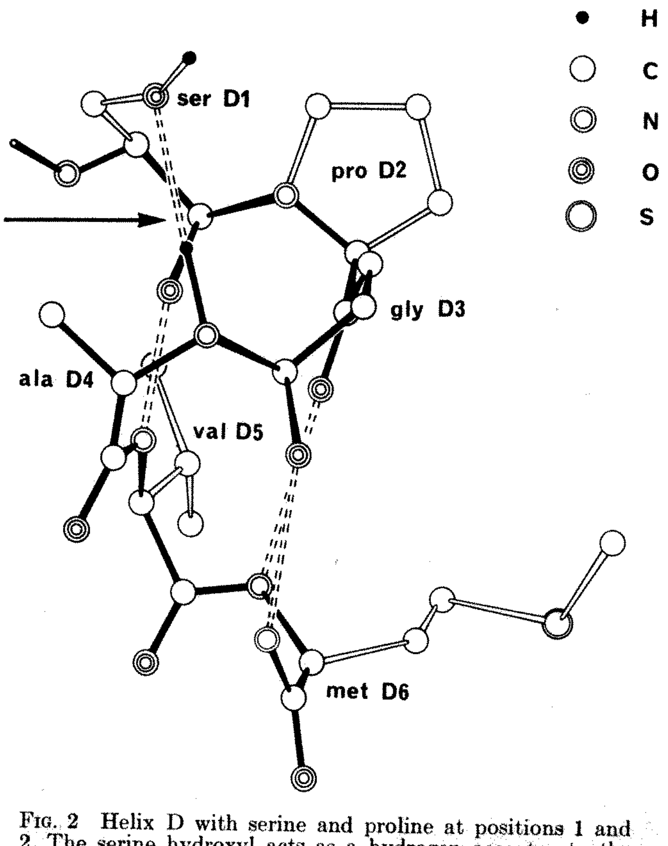

Fig. 2 Helix D with serine and proline at positions 1 and 2. The serine hydroxyl acts as a hydrogen acceptor to the main chain imino group of alanine D4. The hydrogen bond is marked by an arrow. Ionisation of the hydroxyl group would strengthen this bond.

human adult and foetal haemoglobin. Its sequence differs from that of the human adult form in only nine positions in the α and eleven in the β chain[25]. All but four of the twenty replacements are external; one lies in a surface crevice and involves the replacement of Ala G18α by Cys, but the side chain is so positioned that the S^- ion would be accessible to solvent and should not affect the rate of alkali denaturation. Another lies in a surface crevice at GH1 and involves the replacement of Leu by His which would both be uncharged at alkaline pH. Of the two internal replacements, one is Ser for Val at H6β which would both be uncharged below pH 13 and the other is Val for Cys at G14β. Cys G11α and Tyr H8β are both present. The same applies to rabbit haemoglobin[22,27], which also has a rate of denaturation intermediate between that of human and bovine.[28] Table 1 summarises these findings and shows them to be consistent with my interpretation.

Among other species sequenced so far Cys G11α is absent only in bovine, sheep and goat haemoglobin, where it is replaced by serine. Cys G14β is present only in primate and dog haemoglobin; the kangaroo has a cysteine buried in position G15β, but this lies away from the $\alpha_1\beta_1$ contact[29]. The sequences indicate that the high susceptibilities to alkali denaturation found in many adult primate haemoglobins and in the dog are exceptional among mammalian species.

Probable mechanism

In normal, homogeneous haemoglobins, the denaturation reaction follows first order kinetics[3,28]. For several reasons, dissociation of the $\alpha\beta$ dimer into monomers is likely to be the rate-limiting step, followed probably by uncoiling of the chains from the carboxyl end.

The linkage between dissociation and denaturation in human adult haemoglobin has already been noted, but does not tell us which comes first. Haemoglobin Rainier (Tyr HC2(145)β → Cys) is a variant in which helices H and F are joined by a disulphide bridge[30,31]. At pH 12.0 this haemoglobin dissociates into monomers and the normal α chains are denatured, while the abnormal β chains remain native[32]. At the same pH human foetal haemoglobin resists denaturation, even though its α chains are the same as those of Rainier. Apparently the presence of two ionisable sulphydryl groups in the $\alpha_1\beta_1$ contact of haemoglobin Rainier and of only one in foetal haemoglobin causes dissociation of the dimer in the former but not the latter. The $\alpha_1\beta_1$ contact links mainly the G and H helices and the GH corners of the two subunits and, as long as it remains intact, it would oppose uncoiling of the chains from the C terminus but not from the N terminus. The same is true of the disulphide bridge which links residues 93 and 145 in haemoglobin Rainier. Uncoiling from the C terminus may be driven in part by ionisation of the penultimate tyrosines HC2. On the other hand, there are certain serines and threonines whose ionisation would actually stabilise the N termini of several helices at alkaline pH. These are external and occur in positions 1 or 2 of α helices; their γ hydroxyls are so arranged that they can act as hydrogen acceptors from the main chain imino groups four residues forward along the helix[2,33] (Fig. 2). They include Ser A1, E1, F2 and Thr H1β and Thr A1, Ser or Thr D1 and Thr H1β or γ. Because the lone pair electrons of these hydroxyls act as hydrogen acceptors, their pKs should be less than the 13.60 measured in N-acetylserine amide. The ionisation of these hydroxyls above pH 12.0, say, would strengthen their hydrogen bonds with the imino groups and oppose uncoiling of the helices from the amino ends.

Figure 3 summarises the equilibrium mechanism, showing how dissociation of the $\alpha\beta$ dimer allows the sulphydryl groups buried at the interface to become ionised and hydrated; the bulk of the hydration shell opposes reassociation so that the equilibrium is shifted towards the monomeric form. Similarly opening of the tertiary structure of the monomer allows the buried tyrosine to become ionised and hydrated, and access of water into the nonpolar interior displaces the equilibrium towards the unfolded form. Alkali denaturation of human oxyhaemoglobin has an entropy of activation of 39 e.u., and bovine one of only 6 e.u. ref. 28, consistent with activation immobilising more water molecules in the human than in the bovine form. In the denaturation of monomers the electrostatic factor mentioned in the introduction may also play an important part, but buried charges speed the rate as the following example shows.

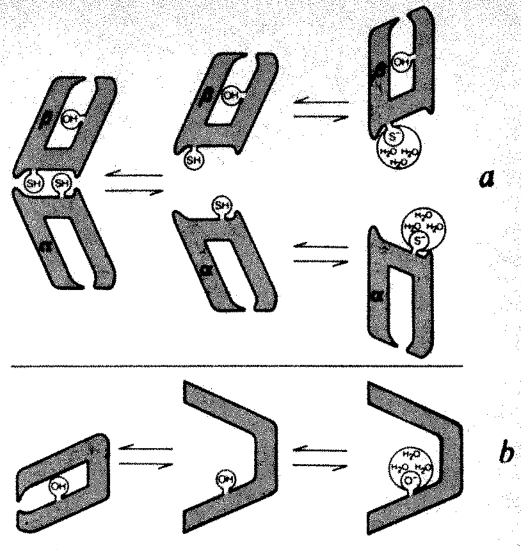

FIG. 3 Hydration effect of buried ionisable groups on the equilibrium between a, dimer and monomers; b, the native and denatured monomer.

The myoglobins of chimpanzee and horse differ in eighteen positions; one of them is G11, buried between helices G and B, which is occupied by cysteine in chimpanzee[34] and by alanine in horse.[35] On reading a draft of this paper, Professor H. Lehmann wondered if this replacement would affect the susceptibility to alkali denaturation. He found that after 20 s at pH 12.7 and 20° C half of the cyanometmyoglobin of the chimpanzee was denatured, but only 10% of that of the horse.

Denaturation of haemoglobin by acid is probably activated by ionisation of histidine G10α which is buried in the $\alpha_1\beta_1$ contact and is invariant in mammals.

I thank Professor N. A. Barnicot, Professor E. R. Huehns, Dr A. R. Fersht and Mr P. A. Lorkin for drawing my attention to published data, and Dr B. S. Hartley for discussion on mechanisms.

Note added in proof. Since this article was submitted, Smith *et al.* have published an observation which bears on the problem discussed here[38]. A human variant haemoglobin has a fused $\gamma\beta$ chain in which the N-terminal half is γ and the C-terminal half is β (ref. 39). It has the same high susceptibility to alkali denaturation as adult haemoglobin A, which proves that this property is determined by residues in the C-terminal half of the β chain.

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Paleomagnetic stratigraphy and chronology of hominid-bearing sediments east of Lake Rudolf, Kenya

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Palaeomagnetic measurements of the polarity of sediments from the Koobi Fora Formation east of Lake Rudolf provide a valuable check on other dating methods and allow a polarity stratigraphy to be suggested for the Formation.

THE Plio-Pleistocene sediments of the Koobi Fora Formation east of Lake Rudolf have yielded a rich assemblage of fauna, hominid fossils and stone artefacts. Here we report a study of the palaeomagnetism of the area, based on over two hundred samples collected under the supervision of one of us (G. Ll.I) and measured in the Nairobi laboratory of the other (A.B.).

Stratigraphy and lithology

Figure 1 is a sketch of the area showing that the accessible outcrops occur along a strip of terrain between an uplifted lava plateau and the modern Lake Rudolf[1]. The sediments rest on lavas, and a block of the underlying volcanics has been uplifted to form the Kokoi structure between Koobi Fora and Ileret. The outcrops of the Koobi Fora Formation with which this report is concerned, have a U-shaped configuration around the Kokoi horst. Palaeomagnetic samples were taken from four stratigraphic columns at points around the U (Ileret areas 6 & 8, the Karari escarpment areas 130 and 131, the KBS Ridge—area 105, Koobi Fora area 103).

Figure 2 shows the columns which have been studied and also a series of tuff layers which are used as marker beds because they were apparently deposited rapidly over wide areas. Potassium-argon age measurements on several of the tuffs suggest that the most reliable determination is for the KBS tuff which yields an age of 2.61 ± 0.26 Myr (ref. 2). The KBS tuff can be recognised in all the main sections including those at Ileret, and it has been used by Bowen and Vondra as a marker for the boundary between the Lower and the Upper Member of the Koobi Fora Formation.

The sediments of the Lower Member are predominantly of lacustrine or lake-margin facies because at the time of deposition proto-lake Rudolf extended much further to the east. They consist of thin bedded clayey siltstones, and silty claystones, with interbedded sheets and lenses of moderately well sorted prodeltaic and littoral sands. The clayey siltstones are reasonably firm and comprise good material for palaeomagnetic sampling.

In area 105 a mappable disconformity has been recognised at a stratigraphic level just above the KBS Tuff[3], and an analagous sharp lithological change with channelling also occurs in area 130. In area 103, in a zone close above the KBS Tuff, there is an episode of tectonic disturbance followed by erosion and the influx of coarser sediments (unpublished work by G. D. Johnson). These disconformities are important for the palaeomagnetic interpretation which will suggest that they represent a time gap as large as 0.7 Myr.

In area 130, the Upper Member consists of comparatively high energy fluvial deposits, with cut and fill bedding in which coarse channel conglomerates and sands pass laterally into flood-plain silts. Only the latter could be used for palaeomagnetic sampling. The Upper Member in area 105 is similar although finer grained. In area 103 the Upper Member is composed of siltstones, mudstones, sand and algal limestones. These represent deposition in littoral and lake margin environments.

At Ileret the sediments above the KBS Tuff are designated as a separate stratigraphic unit called the Ileret Member[1]. The Upper portion of this, which was sampled palaeomagnetically, consists of very low energy fluvial silts and sands with at least one lacustrine incursion towards the top of the section.

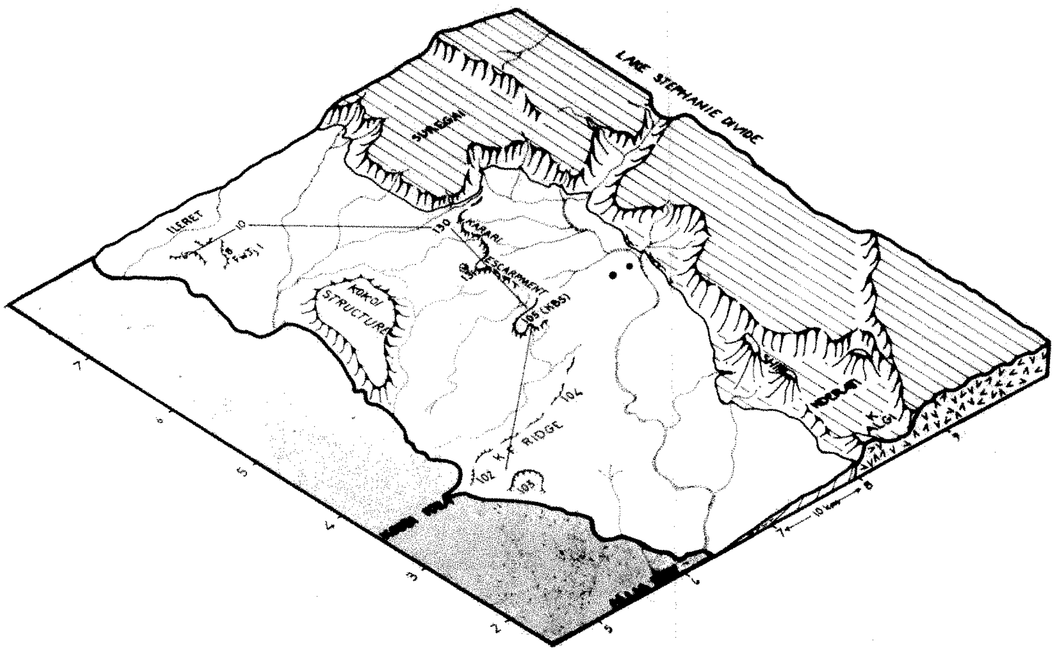

Fig. 1 An isometric view showing the relationship of the studied areas to the modern topography.

Palaeomagnetism

Slumped and weathered material was removed, and non-magnetic tools were used to carve the sediment into small rectangular pillars to fit an open plastic cube of side 2.4 cm. The cube was placed over the pillar and oriented with a compass and inclinometer. The sediment filled cube was then detached and the open side closed with a plastic lid. Wherever possible three or four samples were taken at each horizon, with a spacing of a few metres. Siltstones with varying admixtures of clay or sand were found to be suitable for collection in this way; the coarser grained sediments proved difficult to sample and were avoided. Fortunately, suitable material was abundant in all sections and a total of 247 samples was collected.

The oriented cubes were measured using a 5 Hz spinner magnetometer of the "Foster" type[4], and subjected to the usual a.c. demagnetisation procedures. In most cases secondary components were removed by partial demagnetisation in peak a.c. fields of 100 oersted. Typical demagnetisation curves and directional changes are shown in Fig. 3. An analysis of the directions for information about secular variation, and a study of the magnetic properties of the samples are in progress: here we report solely the polarity results.

The initial directions were classified as normal, reversed, or intermediate (N, R, I). Intermediate directions were defined arbitrarily as those which departed from the geocentric axial dipole field axis for Koobi Fora ($D = 0°, 180°$, $I = \pm 8°$) by more than 50°. The same classification was used for cleaned directions except that in a few cases the cleaning procedure revealed erratic directional changes due to instability. Such samples were described as showing no clear result (X). Table 1 shows the classification of samples before and after demagnetisation. Of the 247 samples, 75 changed their classification after demagnetisation.

The cleaned polarity results are shown on the sampling columns in Fig. 2. The polarities at each sampling horizon are generally consistent. The occasional discrepancies could be due to orientation errors, hard secondary components not removed by cleaning, or discordant ages of magnetisation. Nevertheless the polarity sequence within the columns is generally clear, and the results in Fig. 2 provide the raw

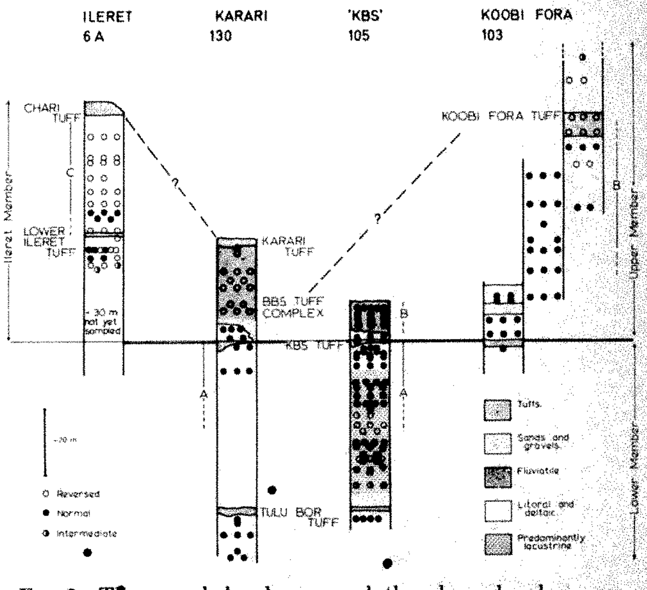

Fig. 2 The sampled columns and the cleaned palaeomagnetic results. Maglio's faunal zones[7] are as follows: A, *Mesochoerus limnetes*; B, *Metridiochoerus andrewsi*; C, *Loxodonta africana*. ○ Reversed polarity; ●, normal polarity.

TABLE 1 Classification of samples before and after cleaning

		After cleaning				
		N	R	I	X	
Before cleaning	N	129	16	8	17	170
	R	0	40	1	0	41
	I	10	20	3	3	36
		139	76	12	20	247

material for making correlations with the geomagnetic polarity time scale. Figure 4 shows the correlation favoured by us. The time scale is based upon that of Cox[5] but the polarity intervals are named according to the scheme of Grommé and Hay[6]. The correlations shown in Fig. 4 are not fully independent, and rely partly upon K.Ar and faunal evidence as well as upon the basic polarity data.

Polarity stratigraphy and the K-Ar and faunal evidence

The starting point for the correlation is the age of 2.61 ± 0.26 Myr obtained by Fitch and Miller[2] from selected sanidine crystals from pumice specimens from the KBS Tuff. The polarity of samples taken from, in, or near the KBS Tuff is everywhere N which is consistent with the quoted age, and in the correlation diagrams the KBS Tuff is placed at 2.61 Myr in the Gauss normal epoch.

The polarity of the Lower Member from the KBS Tuff downwards is predominantly normal, but in area 105 two short reversed intervals occur in a stratigraphic position which suggests that they represent the Kaena and Mammoth events. The sampled part of the Lower Member thus appears to have been deposited entirely during the Gauss normal epoch. In area 105 the spacing of events in relation to each other and to the KBS Tuff implies a net accumulation rate of about 10 cm in 10^3 yr. A similar rate can be anticipated in area 130 and in area 131 (not sampled) which are of similar facies and which show a similar thickness between the KBS Tuff and the Tulu Bor Tuff (50 m).

An independent chronology for the Koobi Fora Formation has been presented by Maglio[7] on the basis of mammal fossil zones, and it is interesting to compare these with the palaeomagnetic results (Fig. 2). Maglio's *Mesochoerus limnetes* zone (A) is defined by an assemblage of fossils from the upper part of the Lower Member which has normal polarity. A 'best fit' age of 2.3 Myr with a range of uncertainty from 3.0 to 2.0 Myr is suggested for this zone on faunal grounds. The palaeomagnetic evidence requires an age greater than 2.43 Myr and in conjunction with the K/Ar determination for KBS Tuff it suggests that the age for the center of the *Mesochoerus limnetes* zone be revised upwards to about 2.7 Myr.

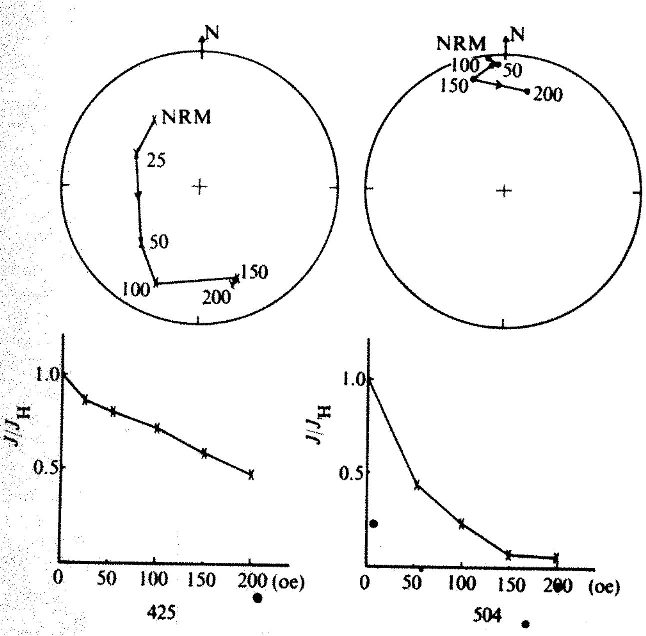

FIG. 3 Typical demagnetisation curves and directional changes for samples **425** (N → R) and **504** (N → N). Secondary components are largely removed by **50** oersted.

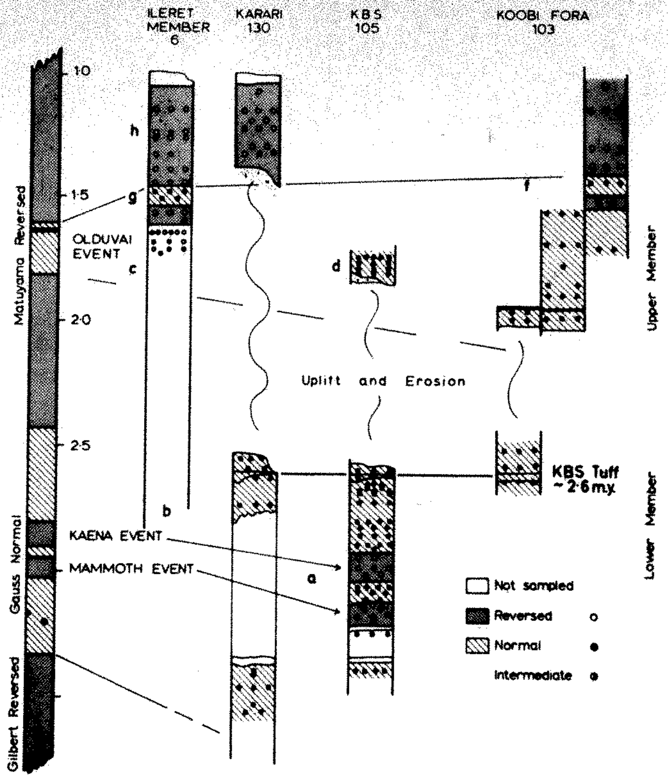

FIG. 4 Tentative correlation of the polarity results with the geomagnetic polarity time scale. The letters a-h refer to the groups of hominid fossils in Table 2.

The fossils used to define the second, or *Metridiochoerus andrewsi*, zone (B), come from outcrops of the Upper Member in which the polarity is predominantly normal. Maglio's estimate for the age of this zone is 1.7 Myr with a range from 1.9 to 1.5 Myr which would imply that the normal polarity belongs to the Olduvai Event. The recognition of a disconformity just above the KBS Tuff in both areas 103 and 105 fits this interpretation and implies a span of time unrepresented by sediments, of the order of 0.7 Myr. The ragged upwards transition from normal to reversed in area 103 would then mark the end of the Olduvai event.

Maglio's third faunal zone, *Loxodonta africana* (C) is represented only in the Ileret Member above the lower tuff, and has an estimated age of 1.3 ± 0.3 Myr. The palaeomagnetic column starts just below the Lower Tuff with a rather mixed polarity pattern, changing upwards into a consistently reversed section to which the main fossil assemblage belongs. Until this column is extended further downwards its placement in the reversal chronology must remain conjectural, but it seems likely that the magnetically complex zone represents the end of the Olduvai event, with the reversed beds above being in the post-Olduvai part of the Matuyama. This would be in accord with the faunal age.

The Upper Member in area 130 gave consistently reversed polarities from the local disconformity up to the Karari Tuff. Few mammal fossils have been recovered from these beds, although they do contain many archeological sites. Since there are grounds for correlating the Karari Tuff in area 130 with the Chari Tuff in the Ileret area, and since both have reversed polarity a similar, post Olduvai event age, is suggested for this part of the area 130 column.

Relations to palaeo-anthropology

The large number of hominid fossils recovered from some sections prevents us from plotting them directly on the palaeomagnetic stratigraphy diagram in Fig. 4, but the approximate position of sets of fossils are shown on the diagram and Table 2 provides lists of the specimens involved. Lists and brief descriptions have already been published by Leakey[8–11,13]. The table extends the equivalent compilation in Maglio's article (ref. 7, Table 4) by including the 1972 field season collection, and by using the polarity correlations of Fig. 4 to suggest age ranges.

TABLE 2 Relation of hominid fossils to marker beds

Ileret	Karari Escarpment	KBS Ridge	Koobi Fora Ridge		Suggested age (M yr)
Areas 1, 2, 6, 7, 8, 10, 12	Areas 130, 131	Area 105	Area 104	Area 103	
Chari Tuff	Karari Tuff				
h *404* *725* *728* **731** *739* *740* *741* *805* **992** *993* *1463* *1465* **1466** *1467*	Industry II				1.4 – 1.6
Middle Tuff					
g *729* *733* **803** **806** **807** *808* **809** *818* *1468*	BBS Tuff			Koobi Fora Tuff	
Industry II				f *403* **730** **734** *736* **737**	
Lower Tuff					
c *406* *407* *727* *732* *801* *802* 815 *819* **820** *1170* *1171* *1464* **1591** **1592**		d *405* *738* *1476* *1477* 1478 *1479*	e 164 *810* *812* **813** *814* *816* 997 998		1.6 – 1.8
KBS Tuff	KBS Tuff	KBS Tuff Industry I			
b **1590** 1593	Industry I a 1462 *1469* **1470** *1471* **1472** 1473 **1474** **1475** **1481** 1482 1483 *1500*				2.7 – 3.0
	Tulu Bor Tuff	Tulu Bor Tuff		Tulu Bor Tuff	

406 = cf. *Australopithecine boisei*; **806** = cf. *Homo* sp.; 998 = affinities indeterminate.
All numbers are Kenya National Museum East Rudolf catalogue numbers (KNMER). Basic information on the fossils has been published by Leakey and others (refs. 8–11, 13). See Fig. 4 for the relationships of sets a–h to the palaeomagnetic columns.

The oldest group of finds, including the important new cranium KNM-ER-1470[11], come from area 131 (set a). They were collected from outcrops of the Lower Member at a level equivalent to that in which the Kaena and Mammoth events have been identified in the adjacent area 105 and slightly below the most richly fossiliferous beds of the *Mesochoerus limnetes* fossil zone. An age of 2.7 to 3.0 Myr for this group is strongly indicated.

Hominids have also been recovered from the *Metridiochoerus andrewsi* zone, and in areas 105 and 103 the relevant hominid horizons have palaeomagnetic coverage. The Upper Member in area 105 and its fossils (set d) appears to be of Olduvai event age, whilst the hominids from area 103 (set f) were recovered from a horizon close to the change-over from N to R, presumed to mark the end of the Olduvai event. An age of 1.6 to 1.8 Myr is therefore suggested for this group. Fossils from between the KBS Tuff and the lower tuff within the Ileret Member (fossil set c) are probably of similar age, but the palaeomagnetic context of these fossils has not yet been determined in full.

At Ileret the beds above the Lower Tuff have yielded numerous hominid fossils (sets g and h). These beds belong to the *Loxodonta africana* zone and having reversed polarity they are presumed to post-date the Olduvai event. An age range of 1.4 to 1.6 is likely for these fossils but can not be regarded as confirmed until the column has been extended downwards.

The Koobi Fora Formation has also yielded a number of artefact sites. The oldest of these, shown in Table 2 as "Industry I" are closely associated with the KBS Tuff and have already been described[3,12]. A younger series of sites, "Industry II" have now been discovered in the Upper Member of area 130 and in the Ileret Member. This industry is contained in beds with consistently reversed polarity. An upper Matayama age is likely—perhaps about 1.5 Myr.

It must be emphasized that although the results shown in Fig. 2 are firm, the correlation suggested in Fig. 4 is still tentative. Because the area is not yet sufficiently densely sampled it is possible to propose other positions for various parts of the columns. Thus the blocks of normal polarity which are here assigned to the Gauss epoch might possibly be part of the Olduvai event. But each variation raises problems, and in our view the correlation proposed in Fig. 4 best satisfies the present evidence. The most secure correlation is that for the lower Member between the KBS Tuff and the Tulu Bor Tuff, the least secure is probably that for the Ileret Member. Nevertheless we feel that the palaeomagnetic results reported here demonstrate the potential of the method, and already provide a basis for extending and checking other dating methods.

Since this paper was written we have seen unpublished results from the F. J. Fitch–J. A. Miller dating group which reports a number of new isotopic ages for the main marker tuffs. It is interesting to compare our suggested placements for the tuffs with their ages; in every case the isotopic and palaeomagnetic ages are consistent, and we believe that this independent evidence greatly strengthens our proposed chronology.

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Magmatism, orogenic timing, and orogenic diachronism in the Cordillera from Mexico to Canada

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The distinction between the largely intra-Cretaceous Sevier orogeny and largely post-Cretaceous Laramide orogeny in the Cordillera between Montana and New Mexico does not exist in the northern Rocky Mountains of Montana and Canada and in the Cordillera of Mexico. An explanation of this in terms of the tectonics of the region is proposed.

THE region of high heat flow, and consequently thin lithosphere, behind active volcanic arcs is of profound importance in the tectonic evolution of continental margin orogenic belts. Armstrong and Dick[1] discuss how overthrust sheets of crystalline rocks are dependent for their genesis on such regions of anomalous heat flow, and it is possible to adopt the even more generalised hypothesis that crustal weakening in zones of anomalous heat flow is a necessary precondition for any sort of tectonic deformation resulting in a large-scale crustal-shortening strain within lithosphere plates. Normal lithospere (whether continental or oceanic and far from rises or hot spots) with a near-surface thermal gradient of less than 30° C is presumably exceedingly difficult to deform by compression. Stresses within the lithosphere will find their release along plate margins and in weaker zones whenever possible, and any region of high heat flow is obviously vulnerable.

In the Cordillera of western North America, nature has performed a grand, controlled experiment to test the hypothesis that behind-the-arc heat flow controls orogenic deformation. A fortunate by-product of this viewpoint and discussion is an explanation of tectonic diachronism along the Cordilleran fold and thrust belt that separates the flat-lying strata of the craton from the more complex structural belts that lie furher west.

The problem of diachronism and a possible explanation have come from two seemingly divergent directions of research—radiometric dating of Mesozoic and earliest Tertiary igneous rocks related to volcanic arc activity, and stratigraphic bracketing of deformation in the fold and thrust belt, which for most of its length lies east of and is geographically distinct from the shifting belt of igneous activity. Here I am not concerned with the complex and incompletely deciphered events of more recent date than early Eocene, the time when a major change in Cordilleran tectonic patterns occurred[2].

King[3] recognised the significant changes along regional strike in the Cordillera and proposed a subdivision into segments. In the area discussed in this paper he recognised four such segments: Mexico, California-Colorado, Oregon-Montana, and Alaska-Canada. The boundaries of the California-Colorado (C-C) segment are shown in Figure 1. For my purposes it is only necessary to distinguish that segment from areas to the north and south.

The obvious distinction of the C-C segment is the eastern Rocky Mountains—uplifts of Precambrian basement bounded by steep, reverse, and thrust faults. Thin sediment cover here was deformed in passive response to movements of the crystalline basement or became detached in gravity-driven slide masses. These Laramide structures, including the Laramie Range itself, can be precisely dated because intervening basins preserve a complete record of their evolution; from first appearance very late in Cretaceous (late Campanian, ~ 72 m.y. ago) time to end of active rise in early Eocene time (~ 52 m.y. ago)[4]. Contrasting in time of deformation and tectonic style is the Sevier orogenic belt[5–7] of this same sector where miogeosynclinal sediments were deformed by low angle thrust faults and folds of décollement style. Preservation of orogenic clastic rocks in Utah and Wyoming makes possible the dating of most of the fold and thrust structures as Cretaceous. Movement began possibly as early as latest Jurassic time (145 to 150 m.y. ago) and had largely ceased in central Utah by late Cretaceous (late Price River—late Campanian, ~ 75 m.y. ago) time, although some deformation persisted into the Palaeocene, resulting in folding of conglomerates of the Flagstaff Formation. Likewise, most of the deformation in Wyoming preceded deposition of the Evanston Formation (Maestrichtian, ~ 65 m.y. ago) although some modest displacement on thrusts did continue

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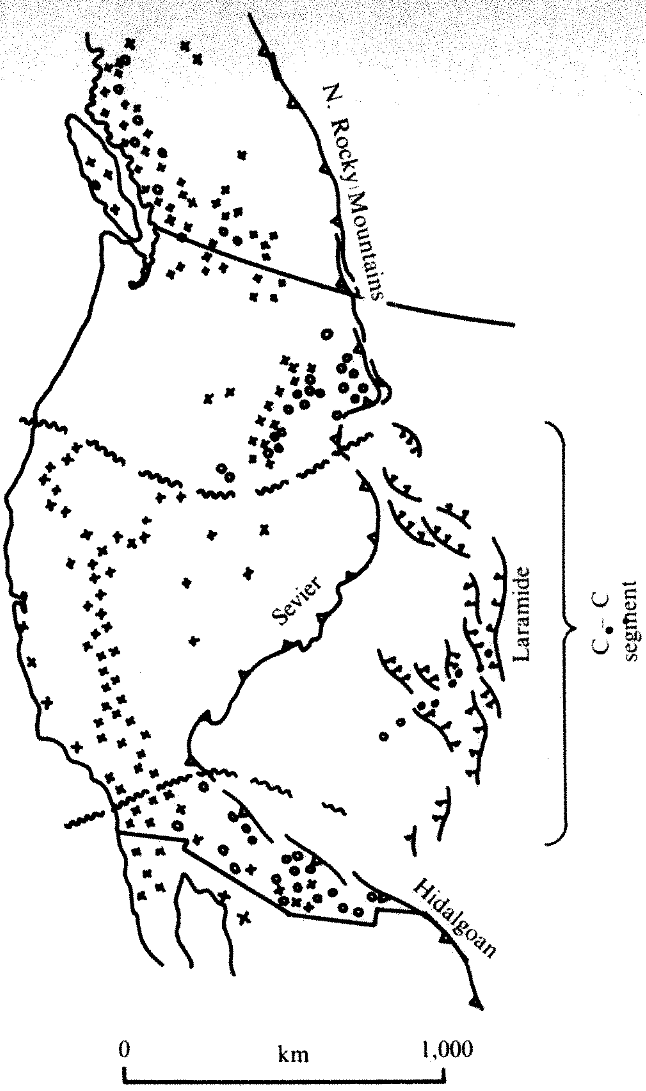

FIG. 1 Index map of central western North America showing the extent of the California-Colorado segment (C-C) and structural belts discussed in the text. X, Generalised distribution of Mesozoic plutons older than 80 m.y.; O, plutons younger than 80 m.y. but older than 55 m.y. Plutonic rocks are shown as they are the prime source of information on the distribution of Mesozoic igneous activity. A one-to-one correspondence between pluton emplacement and volcanism appears to have been true[31] but the preservation of the volcanic record is poor due to erosion. Segment boundary shown as wavy line.

during Palaeocene and up to middle early Eocene time (~ 53 m.y. ago)[8].

This contrast of Sevier and Laramide orogenies does not exist outside of the C-C sector. It has been traditional to apply the term Laramide to the period of deformation of the northern Rocky Mountains in Montana and Canada. This is obviously at variance with the usage of the term in the C-C sector. In the northern Rocky Mountains belt of folds and thrust faults, precise determination of the age of individual structures has been a more frustrating exercise than in Utah-Wyoming. A popular idea was that most of the deformaton was Eocene, and this conclusion was emphatically stated in the literature up to the mid-1960s[9–14]. The available evidence in southern Canada was that strata up to Palaeocene in age were deformed in the Foothills belt; deformation of the Fernie basin in the Front Ranges had to postdate lower Cretaceous time and at least 50 km of displacement is younger than Belly River (lower Campanian ~ 78 m.y. ago) time[15,16].

A marked break separates Palaeocene clastics from upper Eocene to Oligocene post-orogenic deposits, and this was considered by many to be the mark of an orogenic climax.

Acutely aware of the contrast in types of evidence used for dating orogeny in the Rocky Mountains of Canada and the Sevier orogenic belt and the conflicting conclusions reached in the two areas, Price and Mountjoy[17] adopted the position that the Rocky Mountains in Canada were tectonically active from Upper Jurassic to Eocene time. The Jurassic to Palaeocene clastic wedges were interpreted to be a direct record of tectonic activity as the mountains grew and the deformation front moved eastwards—the deformation of Palaeocene strata being the last act rather than the result of a brief catastrophic orogeny in Eocene time. This view put interpretations of tectonic chronology in different segments of the Cordillera more in accord and solved inconsistencies in the Canadian picture such as evidence of lower Cretaceous structures in the western Main Ranges[17].

Evidence of orogenic timing in Montana, obtained during the past decade, is in accord with Price and Mountjoy's view[17]. Most of the deformation of Palaeozoic and Mesozoic strata, including local thick accumulations of Upper Cretaceous volcanic rocks, had taken place before the emplacement of the Boulder and Phillipsburg batholiths (73 to 79 m.y. ago, ~ Campanian)[18–20]. A little further east the deformation occurred even later—after Adel volcanism (Maestrichtian, ~ 65 m.y. ago)[21,22], and after emplacement of late Cretaceous and sills in the Sun River area[23]; movement occurred along the Eldorado thrust after Palaeocene deposition, but prior to intrusion of a 58.3 m.y. sill[24]. In south-westernmost Montana the Upper Cretaceous to Palaeocene Beaverhead Formation was deposited in an area where Laramide style basement uplifts and Sevier style thrusts were concurrently supplying orogenic clastics[25–26]. Most of the deformation of the Beaverhead itself was during the Palaeocene. In the Yellowstone Park region of Idaho, Montana, and Wyoming rocks as old as upper Lower Eocene (~ 50 m.y. old, perhaps as old as 53 m.y.), the Challis and Absaroka volcanics, are clearly post-orogenic (refs 27 and 28 and R.L.A., unpublished).

From this brief review it should be evident that fold and thrust belt deformation is diachronous along as well as across regional strike. Although the Sevier and Canadian Rocky Mountain deformations have beginnings that are obscurely dated but seem similar and although the derived clastic wedges show astounding parallelism of development, their terminal phases do not match, regardless of how the data are manipulated or stretched. Deformation involving tens of kilometers of shortening occurred in Montana and Canada, while the movements in Utah and Wyoming were dying out. The orogenies cannot be equated or used as synonyms. If anything is true, Canadian Rocky Mountain orogeny equals the Sevier plus Laramide orogenies in time. A significant shift in locus of crustal strain that took place in the C-C sector about 75 m.y ago did not occur in the sector to the north.

The record of orogeny in the Mexico segment, likewise, does not match that in the C-C sector. The main deformation may even be slightly different in age from orogeny in Canada but the available dating information is not accurate enough to prove that point. Deformation in western Mexico is known to have occurred during much of Mesozoic time, but an exact chronology is not known. A late Cretaceous to Palaeocene clastic-filled foredeep, which is analogous to the foredeep in the C-C and Canadian segments, covered much of eastern Mexico. The orogenic structures supplying the clastics have not yet been identified and named. These clastic deposits were folded in the Hidalgoan orogeny[29,30], which is considered to be early Eocene in age (roughly equated with Laramide). Post-orogenic deposition began in mid-Eocene time.

The Hidalgoan orogenic style is that of a fold and thrust belt. The effects of this orogeny are widespread, all of eastern and southern Mexico being involved. Here as in Canada, Sevier-style orogeny continued through Laramide time. The abrupt eastward shift of the zone of deformation occurred about 60 m.y. ago, apparently not synchronous with major shifts in tectonic pattern in the more northern segments.

At least in the C-C sector (where the necessary documentation is most complete) it is possible to argue for an action-response relationship between magmatic activity in the volcanic-plutonic arc along the continental edge and deformation of the miogeosyncline. Armstrong and Suppe[31] reviewed the magmatic history of the western United States south of 42° N. Acceleration, if not a new beginning, of igneous activity occurred in Upper Triassic time and activity persisted through the remainder of the Mesozoic, reaching a leisurely culmination in the late Jurassic-earliest Cretaceous and a more pronounced culmination in Middle Cretaceous time.

Except for the linear chain of Laramide volcanic centres that diagonally crossed the Colorado Rocky Mountains, magmatic activity in the C-C segment terminated abruptly about 80 m.y. ago.

Soon after each upsurge in magmatic activity in the Andean-type volcanic arc that stretched for thousands of kilometres parallel to the continental margin, deformation advanced eastwards, engulfing ever more of the previously stable miogeosyncline and platform. The upper Triassic beginning of magmatism was followed by orogenic deformation in western Nevada-southern California dated as pre-Upper Jurassic in the Inyo Mountains[32,33], as pre-Lower Jurassic in the Clark Mountains[34] and as pre-Upper Jurassic in northern Nevada[35]. The Upper Jurassic and mid-Cretaceous maxima of igneous activity correspond to the beginning and most intense phases of the Sevier orogeny, respectively, and were synchronous as well with blueschist metamorphism and subduction in coastal California[36]. The abrupt cutoff of magmatism about 80 m.y. ago in the Sierra region was soon followed by the shift from Sevier to Laramide styles of deformation. Rather than coincidence, this is thought to be a cause-effect relationship. Presumably, after igneous activity of the arc had ceased, the anomalously high heat flow that existed behind the arc died out. One could even imagine that the cutoff effect would migrate away from the volcanic front as the response of deeper zones would be more sluggish than shallower ones. Without the high heat flow, the lithosphere behind the arc would thicken, become more resistant to deformation, and orogenic deformation would cease. This would explain the end of the Sevier orogeny, but what about the Laramide and continuation of Sevier-style deformation in areas north and south of the C-C segment?

This is nature's controlled experiment. Both north and south of the C-C sector igneous activity persisted until 10 to 20 m.y. later, the 'Laramide' activity of Arizona-New Mexico and Mexico continuing on into the earliest Tertiary (until ~ 55 m.y. ago) and activity in Idaho-Montana and Canada dying out about the end of the Cretaceous (~ 65 m.y. ago). In both areas where magmatic activity continued unabated, so did Sevier-type orogenic deformation. Only in the C-C sector with its early magmatic cutoff did the change from Sevier to Laramide styles occur.

Exactly what caused the Laramide deformation is still not completely explained, but the discussion and model experiments of Sales[37] make it clear what happened in Colorado and adjacent states was compression, buckling, and shear that deformed the whole crust. The deformation style expresses much less ductility of the lithosphere than is evident in the hinterland of Sevier-style belts. The magnitude of crustal shortening actually evident in the Laramide structures is vastly less than that which seems to have occurred synchronously in the Cordilleran segments that lay to the north and south.

The Laramide structures represent the reluctant and only partial response to stress imposed on the C-C segment by continuing Sevier-style deformation in the Canadian-Montana and Mexico segments. Premature cutoff of arc activity in the C-C segment (an observation for which a precise plate tectonic explanation is not yet available) made it no longer possible for Sevier-style crustal shortening to occur. Compressive stress continued however, and found its release in the Laramide style of orogenic strain. By late Lower Eocene time, ~ 53 m.y. ago, a shift to an altogether different tectonic pattern had taken place in the C-C-Montana-Canada segment; the change in the Mexico segment came soon after that.

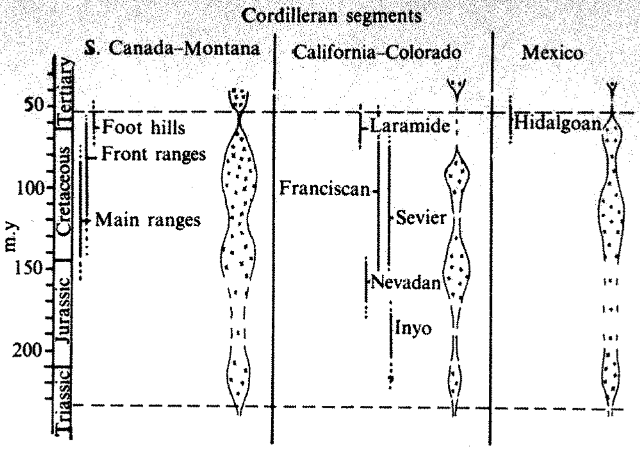

FIG. 2 Summary of chronological information as discussed and cited in the text; duration of orogenic deformation shown by dark lines, arc magmatism of varying intensity suggested by areas with cross pattern. Time scale from Armstrong and Suppe[31].

The change from subduction to a different tectonic style in coastal California occurred in early Tertiary time about the end of the Laramide orogeny, but there is some disagreement among geologists working there as to the exact dating of this event: post-Maestrichtian, pre-mid-Eocene[38]; pre-Oligocene[39]; post-Palaeocene and early Eocene[40]; post-Maestrichtian, pre-late Palaeocene-early Eocene[41]. A portion of the uncertainty may derive from diachronism along strike just as observed in the fold and thrust belt. Subduction that occurred after Santonian time was evidently insufficient to maintain volcanic arc igneous activity. Whether these late stages of movement were intermittent or merely slow and leisurely is yet unknown. What is significant is that compressional tectonics ceased on both sides of the Cordilleran orogen approximately simultaneously.

To summarise briefly (Fig. 2), we have described how the C-C sector of the Cordillera is unique in having overlapping yet distinct Sevier and Laramide orogenies on its eastern side and likewise unique in an early termination of Mesozoic volcanic-plutonic arc magmatic activity in a zone parallel and closer to the west coast. This suggests a cause and effect relationship between an active volcanic arc of Andean type and the orogenic zone far inland from the arc. This is presumably because high heat flow behind the arc weakens the lithosphere so that it may be compressed or stretched apart[42], depending on plate motions dictated by processes acting on a global scale[43]. The anomalous heat flow is not itself a cause of deformation, just a control on where and when it will occur.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Is Cyg X-1 a neutron star?

We suggest that the source of X-ray emission Cygnus X-1 may be a neutron star in orbit about an early-type relatively unevolved star, both of which are in orbit about the massive 09.7Iab (ref 13) supergiant star DH226868. From an analysis of HD226868, Bolton[1] derives the following parameters for the system: $M_1 \sim 20M_\odot$, $M_2 \sim 11M_\odot$, $A \sim 3 \times 10^{12}$cm, $i \sim 30°$, $P \approx 5.6$ d, $L \sim 10^{39}$erg s^{-1} and $T \sim 30{,}000$ K, where M_1 and M_2 are the masses of the primary and secondary components respectively, A is the separation of the centres of the components, i is the inclination, P the orbital period, L the luminosity and T the effective temperature. It has been suggested by numerous authors (for example refs 1–3) that the source of the X radiation is the unseen secondary, which, since it is massive and since it must be compact to produce X rays, is likely to be a black hole.

The alternative hypothesis that Cyg X-1 = HD226868 is a triple system is worth examination. Indeed Batten[5] has noted that about a third of observed binary systems are in fact triple. Specifically we consider the parameters mentioned above and suggest that the secondary consists of two stars—a $1M_\odot$ neutron star (the source of the X rays) and a $10M_\odot$ B1V star with radius $R \sim 3 \times 10^{11}$ cm, luminosity $L \sim 3 \times 10^{37}$ erg s^{-1} and temperature $T \sim 25{,}000$ K (ref. 6) (see Fig. 1). Assuming, for simplicity, that the orbits are coplanar, limits can be placed on the size of the neutron star's orbit around the B1V star. Because of the absence of regular X-ray occultations we must demand 9 h $\lesssim P_N \lesssim 3.3$ days, where P_N is the orbital period of the neutron star. For stability of the system, however, we need the ratio of the radii of the orbits to be more than ~3.5 (ref. 7) which implies $P_N \lesssim 1.4$d.

The optical light curve of this triple system is comprised of two main components. First, there is an underlying ellipsoidal variable contribution, with period $P = 5.6$ d and amplitude of about 0.05 mag in the B band, caused by the distortion and rotation of HD226868 (refs 8 and 9). Second, there is a contribution with periods P and P_N due to the reflection effect (X-ray heating) on both stars. The strength of this modulation depends on the sizes of the orbits and on the total intensity of the X-ray source, but can be comparable to the ellipsoidal effect. Such possibilities can qualitatively explain the anomalous light curve behaviour found by Walker[4] and Martynov[10] especially with regard to the occasional 'filling in' of the secondary minimum.

We now discuss further predictions of the model. Due to the triple nature of the system we expect the radial velocity curve of HD226868, with period P and amplitude ~70 km s^{-1}, to contain a superimposed variation with period P_N and amplitude $\ll 1$ km s^{-1}. The present data are of insufficient accuracy to detect this.

Since the two non-degenerate stars are both early-type, they may both have strong enough stellar winds to power the X-ray source. If the supergiant provides most of the mass transferred, the intrinsic luminosity of the X-ray source would vary in a regular manner with period P_N, unless the time taken for accreted material to lose its angular momentum via the accretion disk is much larger than P_N. In addition, absorption by both stellar winds may provide a quasi-regular modulation of the softer end of the X-ray spectrum[11] with predominant period P or P_N, depending on the relative importance of the winds. The overall temporal behaviour will be complicated by irregularities in the winds and the presence of gas streams.

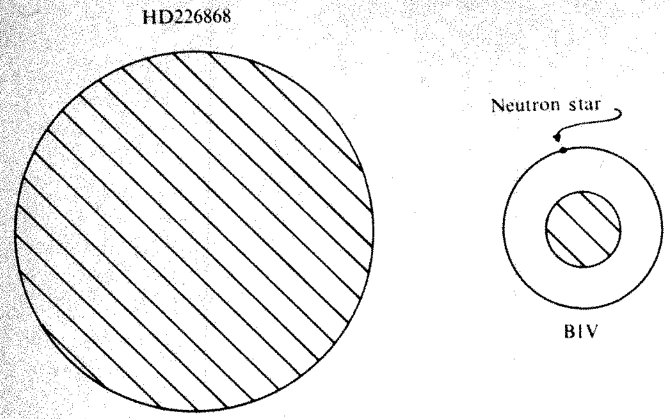

FIG. 1 The system Cygnus X-1 = HD226868 drawn to scale (except for the neutron star). Tidal effects have been ignored.

The X-ray flux from Cygnus X-1 is observed to vary on short (a few milliseconds) timescales[12], but with no regular period. Accretion by a neutron star need not give rise to any regular pulsation, for example if the star's magnetic field is weak ($\lesssim 10^9$ gauss) or if the rotation and magnetic axes are aligned[3]. We note that weak pulsation would be relatively difficult to detect due to the possible high orbital velocity of the neutron star (~ 500 km s^{-1} if $P_N \sim 9$ h). We emphasize too that the shortest timescales of variability to be expected from matter accreting onto a $10 M_\odot$ black hole or a $1 M_\odot$ neutron star are comparable.

We conclude that there is, as yet, no need to invoke the existence of black holes to explain the properties of X-ray sources.

We thank Drs S. J. Aarseth, W. D. Evans and E. N. Walker for help. J. E.P. acknowledges an SRC studentship.

Note added in proof: Professor M. J. Rees has recently drawn our attention to a preprint by Professor J. N. Bahcall and colleagues at Princeton which discusses the possibility of Cyg X-1 being a triple body system and reaches similar conclusions to our own.

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Seismic Wave Scattering Near Caustics: Observations of PKKP Precursors

ANALYSIS of precursors to PKP[1,2] has provided evidence for the existence of scattering structures in the deep mantle. It has been argued that the observability of the scattered waves is closely related to the presence of a caustic because of the focusing effect and because the scattered waves can enter the shadow zone at the concave side of the caustic, where they may manifest themselves as first arrivals. If that interpretation is correct, then in suitable circumstances the scattering phenomenon should also be identifiable near other caustics. The Earth's core forms a caustic not only for PKP phases, but also for SKP, PKKP, SKKP etc. and thus there is, in principle, the opportunity to test the interpretation. In practice there are a number of limitations: first, the latter phases themselves are one or more orders of magnitude smaller than PKP; second, at the time of arrival of these phases the seismic traces are, in general, still 'noisy'. This practically excludes identification from single traces. We have used data from the Norwegian Seismic Array (NORSAR) and here observations in the shadow of the PKKP caustic will be reported. The PKKP caustic agrees mostly with the one of PKP; both caustics extend throughout the whole mantle, in contrast to those of, for example, SKP and SKKP.

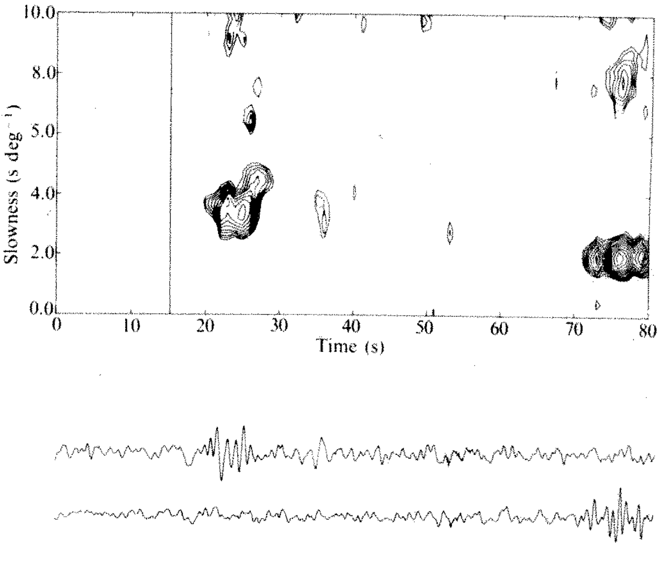

Fig. 1 A Vespagram processed to record PKKP phases from an event with origin time = October 27, 1971, 17 h 58 min 36.9 s, magnitude = 6.0, depth = 40 km. Epicentral distance from NORSAR = 131.6°. The Vespagram start time is at 18 h 26 min 30 s. Power is contoured from 1 to 12 dB below peak power, which is at 77 s. The vertical line at 15 s represents minimum arrival time for PKKP scattering from this event. The traces below the Vespagram represent the NORSAR beams directed towards the PKKP peak (lower trace) and its peak precursor (upper trace).

In detection and identification problems like that it is often advantageous[3,4] to form a display of signal energy versus $dT/d\Delta$ and travel time, called a Vespagram. A typical Vespagram is shown in Fig. 1. It is processed to pick out the PKKP phases from an event at an epicentral distance of 131.6°. The PKKP caustic at the Earth's surface is near 125° and a shadow region for PKKP outer core phases extends from 125° to larger distances, only PKKP which is reflected by and/or refracted through the inner core will be expected here. In the Vespagram it is easy to identify as the inner core phase the peaks in the end of the time window at about 2.0 s deg^{-1}, and it is clear then that there are one or more precursors, starting about 50 s before PKKP. As indicated in Fig. 1, the minimum travel time for scattered waves is at about 15 s after Vespagram start time.

PKKP with its precursors from eight events, all in the Solomon Islands region, have been analysed this way. In the Vespa process the array is steered in a fixed azimuthal direction, which for PKKP is taken the azimuth away from the event (the 'back azimuth'). Since scattered waves may arrive from a variety of azimuths, the maxima in the Vespagrams have been relocated in frequency-wave number space, using the spectral analysis method from ref. 2. Several of the events were fairly complicated, which will cause large arbitrariness in interpreting later arriving precursors. Therefore, in Fig. 2 only results for travel time and direction of approach of the first arriving precursor of each event have been given. In Fig. 2*a* the travel time differences with PKKP have been used, they are plotted along with the PKKP branches from a model of Buchbinder[5]. With these results the following comments can now be made.

(1) It may be remarked that interpretations of PKP precursors in terms of layering in the outer core also predict PKKP precursors. But whereas these interpretations were made to contain the travel times of precursors to PKP, the corresponding PKKP precursors will be 5 to 10 s late with respect to the arrivals in Fig. 2*a*. Thus the 'layering hypothesis' leads here to a discrepancy not only in $dT/d\Delta$ (this would be below 3.3), but also in travel time.

(2) In our working model, $dT/d\Delta$ of PKKP at cusp B is about 4.18 s deg^{-1}. Pursuing now the scattering hyopthesis, phases with larger $dT/d\Delta$ are most likely to be scattered in the deep mantle at the source side of the PKKP path, and scattering characterised by smaller $dT/d\Delta$ should be generated at the receiver side (see ref. 2). With such a model, the travel times are reasonably consistent with the slowness vectors, with a discrepancy of several seconds in only two cases. With one exception, scattering within a few hundred km from the core-mantle boundary can explain the data. This is of course to be expected for first arrivals. Besides, it was argued in ref. 2 that deeper structures are more likely to be revealed, since the direction of scattered waves into the shadow will deviate increasingly from the primary wave direction, if the source of scattering is moved upwards from the core-mantle boundary. Here, this tendency is reinforced by the transmission of PKKP through the core. Although the details of the core-mantle boundary, and especially the density ratio, are still not well known, all plausible models give a high transmission only for PKKP with near grazing incidence from the mantle into the core, which is mainly due to the reflection at the core-mantle boundary within the core. This effect was also recognised in observations of phases which are multiply reflected within the core (PKKKP and so on)[6]. With core phases limited within a ray bundle near grazing incidence, the aforementioned direction deviations of scattered waves will become much more pronounced. Knowing also that the focusing of core phases in this ray bundle increases with depth, the result will be to restrict the source for PKKP precursors further to regions mainly near the core-mantle boundary.

(3) With the limited number of data and the known restrictions for PKKP scattering, inferences on the structure are bound to be very speculative. All we can say at this stage is, that the variations in $dT/d\Delta$ and the relatively small azimuth deviations of most precursors give little indication for lateral variation in the scattering structure. Lateral variations have been inferred from observations of PKP precursors[2] and, though not established, it might be possible that there is a correlation with structural variations suggested by characteristics of direct P waves[7-9]. But those variations are not necessarily manifest in the limited region, which could effectively be 'seen' with the given configurations of sources and receiver. We finally mention that the only precursor with a relatively large azimuth deviation in Fig. 2*c* was also the only one whose scattering source could not be near the core-mantle boundary.

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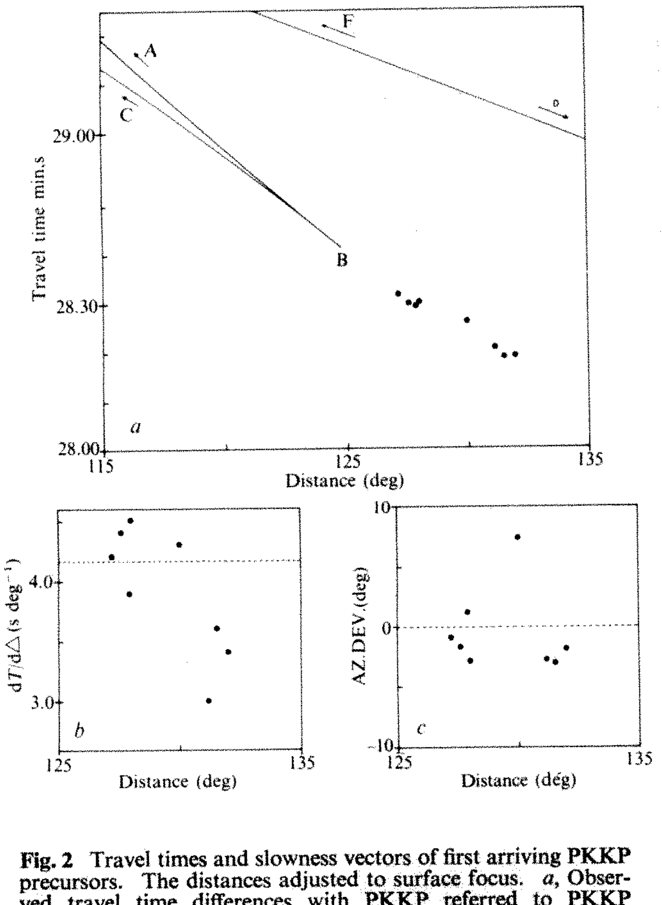

Fig. 2 Travel times and slowness vectors of first arriving PKKP precursors. The distances adjusted to surface focus. *a*, Observed travel time differences with PKKP referred to PKKP branches from a model of Buchbinder[6]; *b*, $dT/d\Delta$ (the line at 4.18 s deg^{-1} represents $dT/d\Delta$ at cusp B for the reference model); *c*, differences between the azimuth of approach and the back azimuth of the event. These differences are called azimuth deviations.

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Age of Fault Movements in Tanzanian Sector of East African Rift System

THE Neogene volcanic province of northern Tanzania is a southerly extension of the more extensive volcanic areas of Ethiopia and Kenya. It stands astride the Eastern Rift Valley and represents a complex interplay of volcanic activity and widespread Earth movements with associated faulting. A general picture is that an older series of basaltic-trachytic shield volcanoes, together with smaller nephelinitic centres, erupted and grew up on the floor of a major late-Tertiary fault-bounded depression; this earlier series includes the basaltic volcanoes of the Crater Highlands, Oldoinyo Sambu, Gelai and Kitumbeine, and the nephelinitic volcanoes Mosonik and Essimingor (Fig. 1). This volcanic area was later disrupted by a major episode of faulting that created the present-day Rift Valley depression. The main manifestation of this faulting is the major Rift Escarpment that runs from Lake Natron in the north to well south of Lake Manyara, this escarpment being the surface expression of the major Manyara–Natron Fault. The Tanzania sector of the Rift Valley is not a classic graben, as in Kenya to the north, and is bounded on its eastern side only by minor faults or down-warping. The major faulting was followed by another phase of volcanic activity, the volcanoes being dominantly nephelinitic, with carbonatite present at some centres; the major volcanoes are Hanang, Ufiome, Meni, Burko, Kerimasi and Oldoinyo Lengai together with many smaller volcanoes[1]. Previous geochronological studies on volcanic rocks from the Tanzania province have outlined the ages of various volcanic centres[2], and also of individual flows in connection with palaeomagnetic determinations[3] or anthropological studies[4]. The work described here has been directed at dating the faulting episodes, particularly the main phase, with a view to setting a maximum age for the onset of the later nephelinite-carbonatite magmatism, and also for the comparison with dated volcanic areas in Kenya to the north[5,6]. Such basic age information is also necessary for examination of the suggested temporal and spatial association of swelling, rifting and volcanicity (pluming?) with carbonatite magmatism[7].

Geological observations suggest that the Manyara–Natron Fault results from two episodes of faulting acting along the same plane of weakness. In the Manyara–Engaruka area, James[8] has recognised an early minor phase—now represented by a mature erosion level at the top of the escarpment—and a later major episode reflected by the steep, less mature lower part of the escarpment. Further north, on

Table 1 Analyses of Rocks Selected from Locations given in Fig. 2

	Sample No.	Locality	Map ref.	K (wt %)	^{40}Ar* ($\times 10^{-7}$ s.c.c. g^{-1})	^{40}Ar*/^{40}Ar$_T$ (%)	Age (m.y.)	Laboratory
Faulting in Tarosero area								
Faulted rocks								
Olivine basalt	309	Tarosero	a	0.520	0.498	4.2	2.40±0.62	S
Sodic trachyte	305	Tarosero	a	4.36	3.928	35.2	2.25±0.06	S
Sodic trachyte	306	Tarosero	a	4.08	3.590	35.4	2.20±0.06	S
Sodic trachyte	510	Tarosero	a	3.82	3.367	22.2	2.21±0.10	S
Unfaulted rocks								
Sodic trachyte	490	Tarosero	b	3.97	3.433*	34.1	2.16±0.06	S
					3.236	71.2	2.04±0.04	S
					3.133	75.8	1.98±0.03	S
Sodic trachyte	488	Tarosero	b	3.86	2.960	50.8	1.90±0.04	S
Main phase of rift valley faulting								
Faulted rocks								
Olivine basalt	304	Matunginini	d	0.686	0.549	4.4	2.00±0.50	S
Olivine basalt	191	Ardai	c	2.85	1.902	13.6	1.67±0.08	N
					2.011	22.7	1.77±0.11	N
Olivine trachybasalt	139	Narabala	e	2.86	1.418	41.4	1.24±0.03	S
					1.367	29.6	1.20±0.04	S
					1.343	25.1	1.18±0.05	S
Unfaulted rocks								
Olivine trachybasalt	137	Narabala	e	3.34	1.616	25.5	1.21±0.05	S
					1.533	30.8	1.15±0.04	S
				3.53	1.533	19.9	1.09±0.05	N
					1.643	31.2	1.17±0.04	N
Olivine trachybasalt	11	Engaruka	g	3.03	1.390	25.4	1.15±0.04	N
					1.342	18.4	1.11±0.06	N
Olivine basalt	181	Kitete	f	0.78	0.351	6.7	1.13±0.16	N
					0.381	10.6	1.23±0.05	N
Olivine basalt	180	Kitete	f	2.42	0.908	12.5	0.95±0.08	N
					1.035	21.1	1.08±0.05	N
Minor phase of faulting on floor of rift depression								
Phlogopite	3	Kisetey	h	7.51	1.71	4.1	0.57±0.15	S
					0.42	1.0	0.14±0.15	S
Phlogopite	891	Loolmurwak	i	7.66	1.63	5.6	0.53±0.10	S
					1.25	4.3	0.41±0.10	S
					0.59	2.7	0.19±0.08	S
Olivine nephelinite	105	Loolmurwak	i	1.36	0.08	1.4	0.14±0.12	S

S, Determined at SURRC; N, determined at University of Newcastle.

$\lambda_e = 0.584 \times 10^{-10}$ yr^{-1}; $\lambda_\beta = 4.72 \times 10^{-10}$ yr^{-1}; ^{40}K$= 1.22 \times 10^{-4}$ g per g K; ^{40}Ar* = radiogenic ^{40}Ar; ^{40}Ar$_T$ = total ^{40}Ar; errors computed from $[E_K^2 + (1 + A/R)^2\ E_{40}^2 + E_{38}^2 + (A/R)^2\ E_{36}^2]^{1/2}$ where $R = (100 - A) = {}^{40}\text{Ar}^*/{}^{40}\text{Ar}_T$ with error in K, $E_K = 1.5\%$ and errors in peak heights, $E_{40} = E_{38} = 0.5\%$; $E_{36} = 1\%$.

* Analysis of powdered sample.

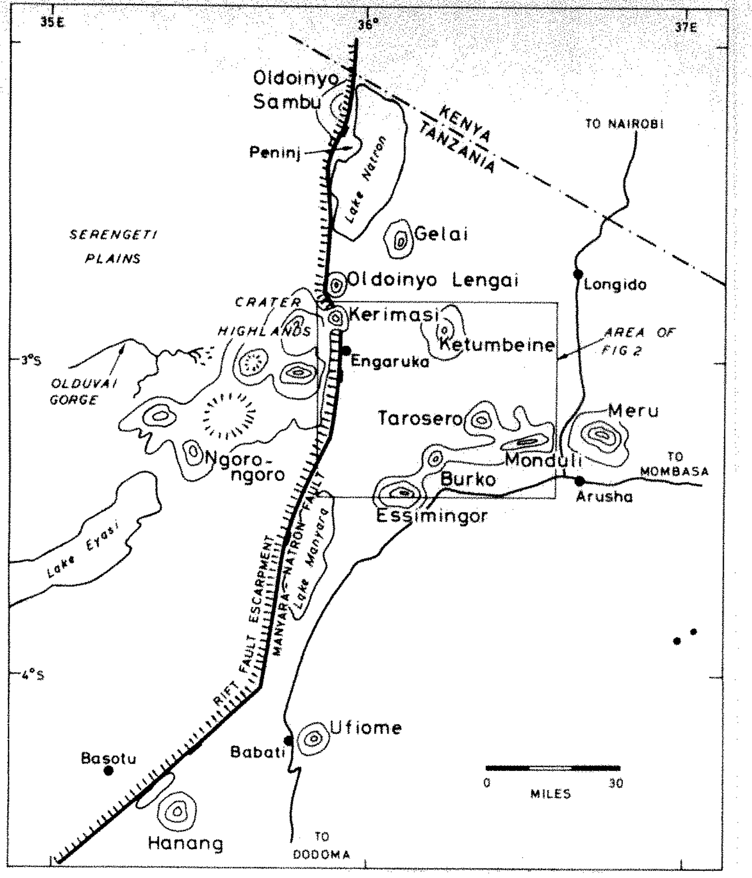

Fig. 1 Map of part of northern Tanzania, showing localities referred to in the text.

the western shores of Lake Natron, the Peninj Group sediments were deposited in a shallow fault-bounded basin that was in existence before the main faulting that has by now exposed these sediments. An early lava near the base of the Peninj sediments has been dated at 2.02 m.y., and olivine basalts higher in the succession (but pre-dating the main faulting) give dates of 1.4 to 1.6 m.y. (ref. 9). An additional minor phase of faulting that post-dates the main faulting has been recognised on the floor of the Rift depression between Engaruka and Lake Natron[10]. These later faults are tightly spaced and of small displacement, and may be analogous to the grid-faulting in the Magadi area just over the Kenya border. Small tuff-rings and cones of olivine-biotite-pyroxene tuffs erupted along these minor faults. The geological evidence shows that eruption occurred after the formation of the Kerimasi volcano but before the initial eruptions of the active carbonatite volcano Oldoinyo Lengai[10].

To determine the age of the various phases of faulting, potassium-argon age determinations have been carried out on three groups of rocks carefully selected for their freshness from a number of critical areas. The rocks were crushed and sieved to $+20$ mesh and the radiogenic ^{40}Ar determinations made by isotope dilution on samples weighing several grams, using highly enriched ^{38}Ar as tracer. Argon isotope ratios were measured statistically using an MS10 mass spectrometer (Scottish Universities Research and Reactor Centre analyses) or an omegatron (University of Newcastle analyses). Potassium was determined by flame photometry on powdered fractions. Previous comparative analyses have shown that no systematic differences exist between measurements carried out in the two laboratories.

The locations of the rocks which were selected for investigation are given in Fig. 2 and the analytical results are presented in Table 1. It is convenient to discuss these results from the three groups of rocks separately and relate them, in turn, to the various phases of faulting.

(1) On the lower northern slopes of the Tarosero volcano (location (*a*) in Fig. 2) faulted olivine basalts and sodic trachytes are exposed in well eroded escarpments resulting from faults of small displacement. In the summit area of the volcano (*b*) these faulted rocks are buried by later extrusions of sodic trachyte. The well eroded nature of the fault-scarps suggests a relatively old age, possibly correlatable with the early movements on the Manyara–Natron Fault. This particular phase of faulting has been bracketed by dating the faulted rocks and later extrusives. The mean ages of the faulted and unfaulted rocks is 2.2 m.y. and 2.0 m.y. respectively, indicating that faulting took place in this area around 2.1 m.y. The ages are similar to those previously reported[2] for the nearby Ketumbeine and Monduli volcanic centres and may closely approximate the beginning of volcanic activity in the area. As the age of faulting is quite compatible with the age for the minor faulting that initiated the formation of the Peninj Basin, it probably also represents the first phase of movement along the Rift escarpment.

(2) Faulted basaltic rocks were collected from the top of younger steep escarpments resulting from the major phase of faulting at localities on the Ardai Plains, west of Arusha (*c*), the Matunginini escarpment (*d*) and at Narabala, on a major splay-fault, which diverges from the Manyara–Natron Fault, south of Engaruka (*e*). The ages measured for these rocks, which range from 2.0 m.y. to 1.2 m.y., are maxima for the age of the major phase of faulting.

At Narabala (*e*) and Kitete (*f*), basaltic rocks have been extruded from fractures resulting from the main faulting and form isolated flows at the base of the fault scarps. In addition, north of Engaruka (*g*) a flow of trachybasalt is interbedded with screes at the foot of the rift escarpment. Age measurements were made on these rocks to establish a minimum age for the major faulting. The ages are mainly around 1.1 m.y. and range from 1.2 m.y. to 0.95 m.y. At Narabala a particularly tight bracket has been found, as the mean of four measurements on two samples of unfaulted lava is 1.15 m.y., and the mean of three determinations on an earlier faulted flow is 1.21 m.y. The combined data indicate that the major phase of rift valley faulting took place between 1.15 and 1.2 m.y. This conclusion agrees well with the evidence that the major Kirikiti–Lengitoto fault (a

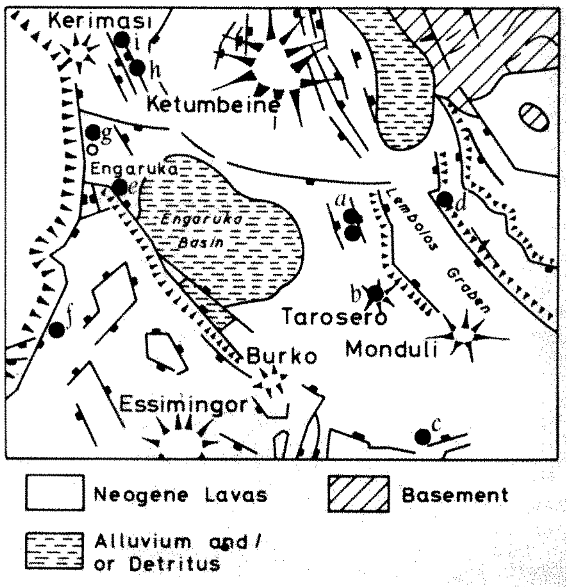

Fig. 2 Map showing localities of samples dated in this work. Localities.● *a*, Lower slopes of Tarosero volcano; *b*, summit area of Tarosero volcano; *c*, Ardai Plains; *d*, Matunginini escarpment; *e*, Narabala; *f*, Kitete; *g*, Engaruka; *h*, Kisetey Hill; *i*, Loolmurwak crater.

major en echelon extension of the Manyara–Natron fault in South Kenya) developed in the period 1.3 to 0.9 m.y. (ref. 5). In addition, the major reversal of drainage and tectonic break between beds II and III of the Olduvai succession, both of which must be the result of some major Earth movement, are dated as post 1.5 m.y. and possibly at 1.0 m.y. (R. L. Hay, personal communication).

(3) The age of the later faulting on the floor of the Rift depression was examined by analysing phlogopite crystals from the tuff-rings at Kisetey Hill (*h*) and Loolmurwak Crater (*i*), as the geological evidence indicates that the faulting and eruption of these two minor volcanic features were probably contemporaneous. A sample of lava from a small lobate flow which has breached the tuff-ring at Loolmurwak was also analysed.

Although the measurements are characterised by a high analytical uncertainty on account of the small concentrations of radiogenic ^{40}Ar relative to atmospheric argon and other gases (2.14% CO_2 by weight in the lava) in the samples, the comparative youthfulness of this volcanic activity is confirmed. The calculated ages range from 140,000 to 570,000 yr and the average age of both phlogopites is around 350,000 yr. The best estimate for the age of eruption which can be derived from the data is about 370,000 yr and the faulting probably occurred near or shortly before this time. This faulting may be of similar age to the grid-faulting of the Rift floor in the Magadi area of Kenya, which has been bracketed between 0.8 and 0.4 m.y. using obsidian dating and pumice and fossil evidence[5]. As the late minor faulting and the main episode of Rift faulting bracket the eruption of the Kerimasi carbonatitic volcano[10], this occurred within the period 1.1 to 0.4 m.y. The age of the minor faulting also places a maximum date of about 370,000 yr for the onset of eruptive activity at Oldoinyo Lengai.

The specimens were collected while one of us (J. B. D.) was a member of the Tanzanian Geological Survey. We thank the Natural Environment Research Council, the University of Newcastle, the University of Strathclyde and other Scottish Universities for financial support during the project, and Mr T. McMenamin for analytical assistance.

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Postspinel Phase of Forsterite and Evolution of the Earth's Mantle

THIS is the first announcement of success in the experimental confirmation of the postspinel phase of forsterite at high pressure and high temperature. Forsterite (Mg_2SiO_4) decomposes to periclase (MgO) and stishovite (SiO_2) at 330 kbar and 1,000° C. Forsterite is a major constituent of the Earth's mantle and the phase of forsterite composition at high pressure places a strong constraint on the nature of the transition layer and also on the composition and evolution of the Earth.

The profile of seismic wave velocity in the Earth's mantle shows two distinct stepwise increases at the depths of 400 and 700 km[1,2]. These steps are thought to be due to the pressure-induced change of phase of forsterite[3], since the Earth's mantle corresponds mostly to forsterite in composition. Thus extensive experimental trials to the phase change of forsterite and similar compounds have been made. The experimental results with forsterite[4–8] are summarised as follows,

$$\begin{tikzcd} \underset{\text{(Olivine structure)}}{\alpha\text{-}Mg_2SiO_4} \arrow[r] & \underset{\text{(Modified spinel structure)}}{\beta\text{-}Mg_2SiO_4} \arrow[d] & \\ & \underset{\text{(Spinel structure)}}{\gamma\text{-}Mg_2SiO_4} \arrow[r] & \underset{(?)}{\text{Postspinel}} \end{tikzcd}$$

in the order of increasing pressure (depth). The transition

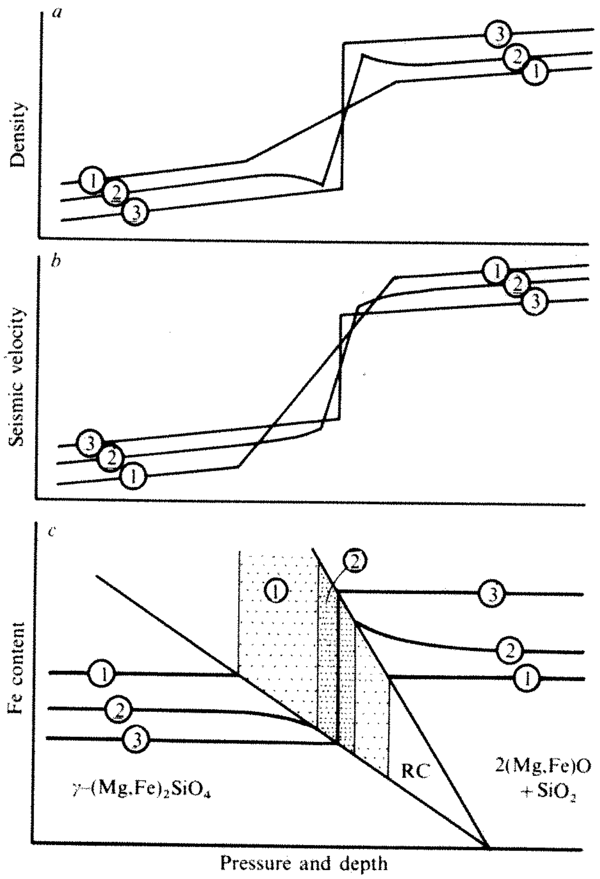

Fig. 1 Schematic drawing of the expected nature of the seismological step at a depth of 700 km. *a*, *b*, Density and seismic velocity profiles. *c*, Phase diagram of forsterite with some fraction of fayalite, and the profile of Fe concentration with depth in the mantle. RC, Region of coexistent phase. 1, Dynamic equilibrium or uniform concentration of Fe throughout the depth range; 3, static equilibrium between the upper and the lower mantles; 2, transitional state between 1 and 3. A sequence 1→2→3 may represent the change of structure of the transition layer at a depth of 700 km.

from olivine to spinel phases (β and γ) is correlated to the seismological step at a depth of 400 km as suggested by Bernal[9]. The seismological step at a depth of 700 km may be due to the transition from the spinel phase (γ-olivine) to a 'postspinel' phase, whose nature is not yet known.

From the physical viewpoint, Birch[3] predicted that the transition would be to dense phases with a closely packed structure. Shimazu and Kumazawa[10] may have been the first to suggest the decomposition to the mixture of MgO and SiO_2. Stishov[11] has shown clearly the possibility of decomposition to periclase (MgO) and SiO_2 with a rutile type structure, which Stishov and Popova[12] first synthesised at high pressure and which was later named 'stishovite'.

Many different possibilities have later been suggested on the basis of the accumulating experimental data on similar and/or related compounds; for example, transition to Mg_2SiO_4 with a strontium plumbate or K_2NiF_4 structure[13], or transition to periclase plus $MgSiO_3$ with an ilmenite structure[14]. No direct experimental confirmation has been made, however, because a method of producing static pressures as high as 300 kbar combined with high temperatures has not been available.

We have been developing an apparatus using the MASS (Multiple–Anvil Sliding System) mechanism[15], a new principle recently devised[16]. By using the apparatus under development, the high pressures up to 500 kbar in a volume of 0.005 cm^3 are successfully reached (Masaki *et al.*, in preparation). The pressure produced is calibrated at room temperature on the basis of resistance measurements of calibrants on the pressure-raising cycle (Bi I–II = 26 kbar; Bi III–V = 76 kbar; Pb I–II = 130 kbar; GaAs semiconductor-metal = 190 kbar; ZnS semiconductor-metal = 190 kbar; CdS resistance maximum = 330 kbar, and ZnS resistance maximum = 430 kbar)[17]. Pressures up to 350 kbar are produced in a pressure medium of 0.02 cm^3, in which a carbon heater and Pt-Pt/13% Rh thermocouple are installed with a sample of 0.2 mg. Temperature is determined by measurements of EMF on the thermocouple without correction for the pressure effect and the surface temperature of the anvils, to which the thermocouple leads are connected. The starting material was a powder of synthetic forsterite (α-olivine), and the product run at 330 kbar and 1,000° C for 30 min was investigated by optical microscopy and X-ray diffraction. Table 1 shows that the product was certainly a mixture of periclase (MgO) (rock-salt structure) and stishovite (SiO_2) (rutile structure). No traces of γ-olivine or of unidentified material were detected.

Table 1 X-ray diffraction data of Mg_2SiO_4 run at 330 kbar and 1,000°C for 30 min ($MoK\alpha_1$)

d_{obs} (Å)	d^* (Å)	Phase	$h\ k\ l$	I_{obs}
3.34	3.35	Carbon †	0 0 2	50
2.960	2.959	Stishovite	1 1 0	65
2.430	2.431	Periclase	1 1 1	20
2.242	2.246	Stishovite	1 0 1	20
2.103	2.106	Periclase	2 0 0	100
—	2.09	Stishovite	2 0 0	—
2.046	2.060	Diamond †	1 1 1	30
1.983	1.981	Stishovite	1 1 1	25
1.867	1.870	Stishovite	2 1 0	15
1.528	1.530	Stishovite	2 1 1	25
1.488	1.489	Periclase	2 2 0	30
1.480	1.478	Stishovite	2 2 0	20
1.338	1.333	Stishovite	0 0 2	10
—	1.322	Stishovite	3 1 0	—
—	1.291	Stishovite	2 2 1	—
1.264	1.270	Periclase	3 1 1	10
	1.261	Diamond †	2 2 0	
1.236	1.235	Stishovite	3 0 1	15

* ASTM Index Card.

† Carbon and diamond: contamination from carbon heater.

A pressure of 330 kbar corresponds to the pressure at a depth of 1,000 km in the Earth's mantle and the transition from the spinel phase to the oxide mixture is correlated to the seismological step at a depth of 700 km, since there is no significant seismological step except a very small one at about 900 km depth in this range. No further phase change in the major constituent minerals is likely to take place in the mantle. Our results therefore suggest that the phase composition in the lower mantle is mainly stishovite and periclase. Of course, periclase should be a solid solution of MgO and FeO. Anderson[18] has already shown that the seismological step at 700 km and the seismic wave velocity in the lower mantle are well explained by the assumption of postspinel phase having the same thermochemical and physical properties as mixture of (Mg,Fe)O and SiO_2.

The thermodynamic calculation shows that the decomposition of γ-olivine into a mixture of oxides is an endothermic reaction[19]. Therefore, the material will meet a large mechanical brake[20,21], when it is going to pass through the horizon of the transition at a depth of 700 km. This explains why the downgoing plate of lithosphere does not sink below 700 km and why earthquakes only occur above this level. In addition, the overall convective motion in the mantle, involving both the lower and the upper mantle, is hardly possible, not simply because of the high viscosity in the lower mantle[22], but because of the mechanical stability originating from the heat and volume balance. This situation places a strong constraint on the evolution of the Earth. For example, if the time of the large scale geochemical differentiation in the Earth was realised through hydrodynamic migration of the material, it should be dated to a very early (unknown) stage of the Earth's history, unless diapirism or similar processes are proved to take place in the lower mantle.

As a straightforward consequence of the static contact of the lower and the upper mantles, there may be partition differentiation of Fe and Mg between the upper mantle (γ-olivine solid solution) and the lower mantle (periclase-wustite solid solution) through a horizon at 700 km. Since the equilibrium pressure of the phases containing Fe is lower than that of pure forsterite, the partition differentiation would result in an enrichment of Fe in the high pressure phase and a depletion in the low pressure phase. Figure 1 shows schematically the density and seismic velocity profiles of the mantle expected from the partition differentiation of Fe and Mg at a depth of 700 km. Even though the primitive mantle is uniform in Fe and Mg concentrations (1 in Fig. 1), the lapse of time will cause the transport of Fe to the lower mantle and that of Mg to the upper mantle (2 in Fig. 1). The migrations of Fe to the deeper part and of Mg to the shallower part in the lower mantle may be due to the diffusion in the gravity field and to the convective overturn of the material driven by the density reversal just beneath the 700 km horizon. In the upper mantle, there are many processes accounting for the mixing and migration of the material as inferred from such phenomena as the observed tectonic movement in the upper layers of the Earth and the expected low viscosity and high temperature gradient. The transition between olivine and spinel at a depth of 400 km is an especially strong factor promoting the migration of materials above and below this horizon, since this transition is unstable[23] due to the exothermic reaction when olivine transforms to spinel. After a sufficently long time, the seismological step at 700 km would become a real first order discontinuity (3 in Fig. 1), which separates the iron-rich lower mantle and the magnesium-rich upper mantle. A notable characteristic of the transition at a depth of 700 km is that gravity differentiation and partition differentiation are completely cooperative. It is a stable interface associated with both a phase change and a chemical discontinuity.

Our interpretation is consistent with the difference between the equations of state on either side of the mantle inferred from the seismological data. When the velocity-density relation obtained by Press[2] is compared with the velocity-density-mean atomic weight systematics by Birch[24], for example, the lower mantle has an apparently higher mean atomic weight, suggesting higher concentration of Fe in the lower mantle than in the upper mantle. It is noteworthy that no inflection is found in the velocity-density relation around a depth of 400 km. In addition, the Fe-rich lower mantle offers a reasonable explanation of the fact that the Fe/Mg ratio in the whole Earth calculated from the present composition of the upper mantle is low when compared with that calculated from chondritic and cosmic abundances. After the formation of the solid Earth, the Fe/Mg ratio in the upper layers of the Earth above 700 km horizon is expected to have been decreasing with geological time by the absorption of Fe into the lower mantle through the horizon at 700 km.

The sharpness of the seismological step at 700 km[25] may be partly due to the negative slope ($dT/dP<0$) of the equilibrium temperature of γ-olivine and (Mg,Fe)O plus SiO_2. It is, however, better accounted for by the equilibrium between the Fe-rich lower mantle and the Mg-rich upper mantle. From our interpretation of the nature and evolution of the transition horizon at a depth of 700 km, the information concerning the sharpness of this seismological step is expected to be a good clue to the processes involved in the chemical differentiation, lateral inhomogeneity of the mantle, and so on. For example, Kanamori[26] has shown that the step at 700 km is unclear beneath the Japanese Islands, making a contrast to that beneath the North American continent[27]. It is likely that the downgoing plate of lithosphere is disturbing the static equilibrium between the upper and the lower mantles.

The experimental details and the results on fayalite and olivine solid solution will soon be reported (Sawamoto *et al.*, in preparation).

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Possible superconductivity at room temperature

IN this paper some observations are presented on an anomalous current in aluminum-carbon-aluminium (Al-C-Al) sandwiches, at room temperature, which in several respects behaves in the same way as the Josephson current might be expected to do. At first the switching effect was studied in Al-C-Al sandwiches discovered by Ovshinsky[1] and Pearson[2] in chalcogenide glasses and amorphous oxides. In carbon sandwiches subjected to proper electrical pulsing, changes in resistance of a factor of 1,000 were found, the changes being reversible and with a memory time of the order of a few days[3].

When more experimental data were collected however, the effect seemed to be more complex. None of the known models proposed for switching phenomena in amorphous materials could be applied to this carbon system[4,5]. Amorphous carbon is so different from materials used in switching devices that the switching in carbon may bear no relation to that in other materials. Most striking was the observation of a strong dependence of the current on a small external magnetic field.

The best results were obtained with samples produced in the following way: the first aluminium electrode was evapo-

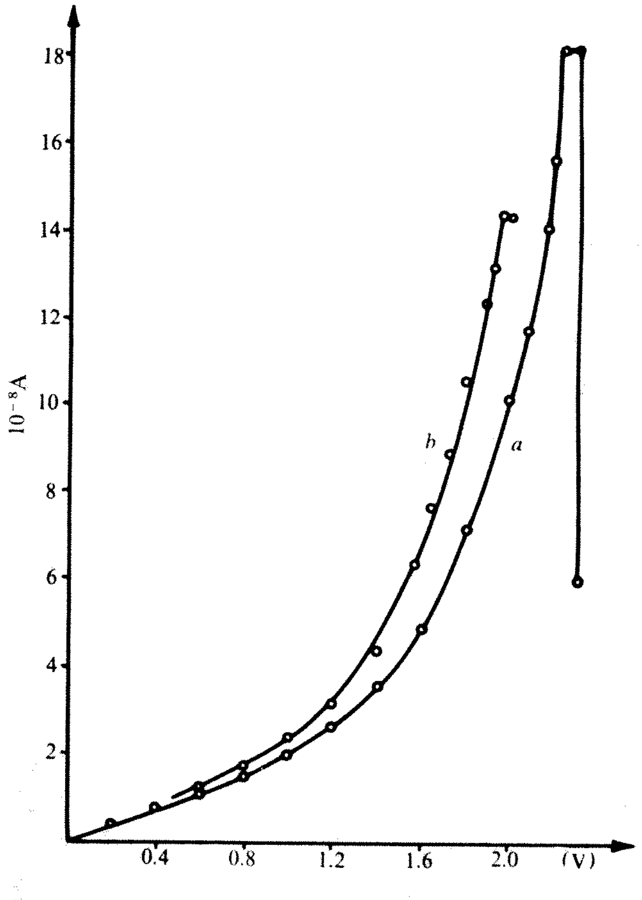

FIG. 1 Current-voltage characteristics for an Al-C-Al sandwich. *a*, Sudden decrease of current after passing J_{max}; *b*, J_{max} approached by a slow increase of voltage.

rated on to a carefully cleaned porcelain substrate. Next the electrode was covered by polyfurfuryl alcohol (prepolymerised to a viscosity of about 10 cpoise and the sample heated up to a temperature of 620° C, the desired temperature being achieved in 80 min. At 300° C, the sample container was evacuated. Following this heat treatment, the upper electrode was deposited.

Current-voltage (I-V) curves were then taken. The current was measured by a mirror galvanometer. The voltage was increased in steps and after each increase of voltage a decrease of current with time was seen. This is probably due to the space charge (the original resistance of the sample is of the order of 10^8 Ω) because, after shorting the circuit, the current flows in the opposite direction like that in a charged condensor. The sample was maintained long enough at each voltage to reach equilibrium.

At about 2 V, a new phenomenon appeared: an increase of current with time at a constant or even decreasing voltage. This generation of conductivity continues for 1 to 2 h and the final current is two to five times larger than that at the beginning. The new state is quasi-stable and in good samples no essential decrease in conductivity can be detected for a few hours, but in a few days it decreases appreciably. Above a particular voltage, further increase in voltage gives rise to a decrease in current (curve *a* in Fig. 1), which is irreversible. To avoid this drastic change in conductivity, the initial voltage was increased gradually until the maximum current was attained (curve *b* in Fig. 1). Once $J_{\max}$ was reached, the voltage was lowered and the measurements were repeated in a new magnetic field. The magnetic field was produced by a coil, both the sample and the coil being inside a Mu-metal cover which shielded the sample from the magnetic field of the Earth.

Figure 2 shows the dependence of $J_{\max}$ on the applied magnetic field. When a field of 0.42 gauss is applied, the current is reduced by a factor of four to a minimum. At each successive integer multiple of 0.42 gauss, the current again goes through a minimum with successively decreasing maxima in between. First reduction of $J_{\max}$ was observed in various samples at fields as low as 0.1 gauss and as high as 1 gauss. While $J_{\max}$ varied by as much as two orders of magnitude.

Strong dependence on the magnetic field is known in tunnelling between two superconductors[6]. The Josephson DC tunnelling current in a magnetic field is given by the formula[6]:

$$J = J_0[\sin(\pi\phi/\phi_0)/\pi\phi/\phi_0] \sin \delta$$

where ϕ is the flux enclosed by the area of the junction; ϕ_0 is the quantum unit of the magnetic flux; J_0 is a constant for a given junction at a constant temperature and δ is the phase difference which adjusts itself to the experimental conditions. As the current is increased, δ changes from zero for zero current to $\pi/2$ for the maximum allowable current. The expected dependence of $J_{\max}$ on the enclosed flux is

$$J_{\max} = J_0[\sin(\pi\phi/\phi_0)/\pi\phi/\phi_0]$$

The Josephson current through a single junction is a periodic function of the flux enclosed in the junction. Such a dependence is the most convincing evidence that the current is a tunnelling current between two superconductors.

It is well established that in switching devices the current flows through a narrow path. The existence of a conducting path in carbon sandwiches can easily be seen when samples are destroyed because of overloading. In such samples, craters or pits in the aluminium electrodes are seen and are attributed to filament path formation. There are indications that in carbon sandwiches these paths are formed by a trail of conducting drops, manifesting themselves when the decay of the conducting state is observed as a function of time. The conductivity does not decay continously but remains approximately constant for a long time then drops rapidly to a lower value, remains roughly constant then drops again and so on. This seems to be a result of the inhomogeneous structure of amorphous carbon.

As far as is known, amorphous carbon consists of small crystallites of graphite-like layers with little layer stacking order and with strong lattice distortion. Studies of small-angle X-ray scattering from glassy carbon show that the impenetrable pore volume is made up of many submicroscopic voids of average pore size about 25 Å. One could suppose that the tunnelling of electrons occurs through those voids but the area corresponding to the periodicity $\Delta H = 0.42$ gauss in the interference pattern is about 5×10^{-7} cm^2. This is very much larger than the cross-sectional area of voids. On the other hand, the high percentage of samples which show the desired effect (about 30% when properly produced) indicates the existence of a natural mechanism producing barriers. One of the possibilities is the cracking of the carbon layer caused by the difference in the coefficients of the thermal expansion of the substrate and of carbon. The under part of the carbon layer adheres tightly to the porcelain, the upper part is free. It may be expected, therefore, that after heat treatment, cracks will always appear inside the carbon layer and that these cracks are responsible for the appearance of barriers.

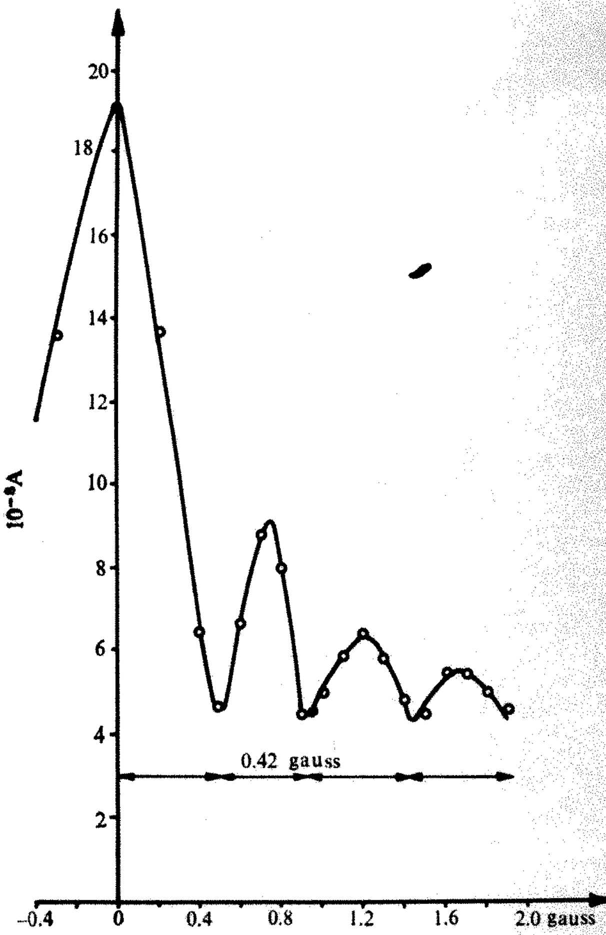

Fig. 2 Dependence of $J_{\max}$ on the applied magnetic field.

A common feature of all the samples investigated is a long relaxation time; the time needed to determine $J_{\max}$ at a given magnetic field is about 0.5 h. Such long time constants are known to appear when deep electron traps are involved and in amorphous carbon a high concentration of such traps is found.

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Tensile Strengths of Carbon Fibres

THE strength of carbon fibres prepared from commercially available polyacrylonitrile (PAN) fibres depends on the heat treatment temperature (HTT) and normally decreases[1] from a maximum after a HTT of about 1,500° C. Moreton[2] found that after all heat treatment temperatures (1,000–3,000° C) the strength is dependent on the gauge length indicative of a random distribution of flaws. Johnson[3] and Thorne[4] demonstrated that both surface and internal flaws are present, the latter due to foreign particles in the PAN precursor fibre reacting with the fibre during high temperature processing. Sharp and Burnay[5] subsequently used high voltage electron microscopy to examine carbon fibres directly and concluded that, when the HTT is raised above about 1,800° C, inclusions react and volatilise to form voids. This results in the formation of misoriented three-dimensional graphite on the walls of the voids and decreases the strengths of the fibres.

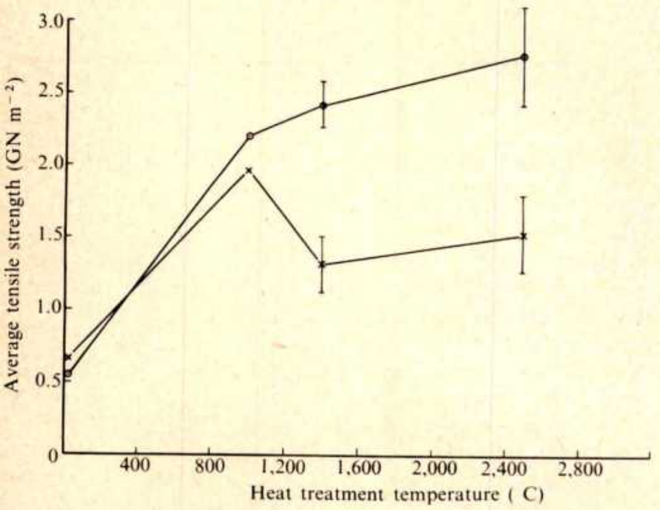

Fig. 1 Effect of heat treatment temperature on fibre strength. Error bars denote 95% confidence limits. ○, Clean room fibre; ×, control.

Attempts have been made to apply the Griffith brittle fracture criterion to carbon fibre strengths. Whitney and Kimmel[6] used maximum flaw dimensions to calculate values for the surface energy and obtained values three to ten times greater than the theoretical value and so suggested that plastic deformation was taking place at the roots of cracks. Sharp and Burnay[5], on the other hand, assumed a value for surface energy and calculated the critical crack lengths to be much smaller than the flaws they had observed. They therefore proposed that fractures were initiated at cracks in the walls of the voids. Coyle *et al.*[7] have also suggested that graphitic regions associated with impurities lead to reduced carbon fibre strengths. Failure mechanisms not involving impurities have also been put forward. For example, Le Maistre and Diefendorf[8] proposed a circumferential arrangement of the layer planes and suggested that circumferential cracks are formed on cooling after the final heat-treatment due to thermal expansion anisotropy. Cooper and Mayer[9], however, thought that cracks were formed as a result of dislocation pile-up with strength being inversely proportional to crystallite size[1].

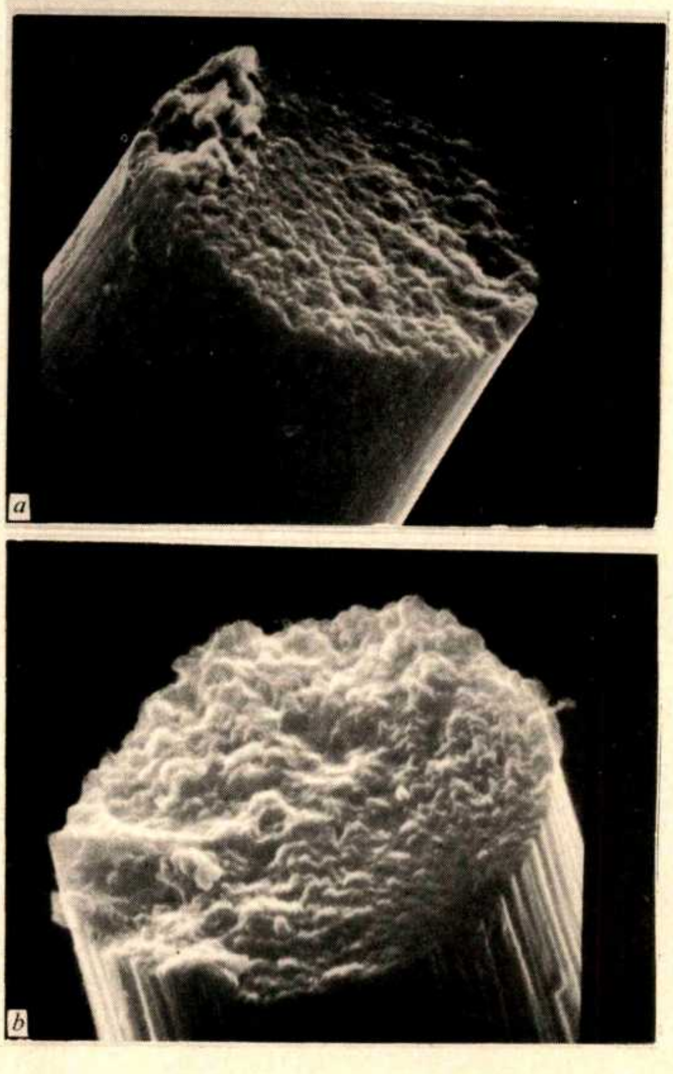

Fig. 2 Fracture surfaces of carbon fibres heat treated to 1,400° C. *a*, Control fibre with diameter 7.5 µm and strength 1.5 GN m^{-2}; *b*, clean room fibre with diameter 8.1 µm and strength 2.6 GN m^{-2}.

In this laboratory it was decided that the effects of impurities must be removed if theories concerning the intrinsic strengths of carbon fibres were to be properly evaluated. Accordingly we have spun PAN fibres under class 100 clean room conditions (US Federal Standard 209A) (<100 particles per foot^3 of air at 0.5 µm and above) from a solution of the polymer filtered through a 1.5 μm filter. The fibres were then processed under clean conditions into carbon fibres using methods already described[10] and heat-treated to 1,000, 1,400 and 2,500° C. Control samples were also obtained by spinning under normal laboratory conditions (approximately 10^6 particles per foot^3 of air at 0.5 μm and above) a similarly filtered solution of PAN and these were converted to carbon fibre in the same way as the clean PAN fibres. Figure 1 shows the effect of HTT on the average strengths of the two batches of carbon fibre, each point representing an average value obtained from twenty tests using 5 cm gauge lengths. The strength of carbon fibres from the clean room precursor continued to increase with HTT whereas the control fibres suffered a marked strength decrease after the treatment to 1,400° C. Thus, after the final heat-treatment to 2,500° C the clean sample had a strength 80% higher than that of the control fibres. A further point of interest is that the strengths of the clean fibres were unaffected by gauge length over the range 1 to 5 cm whereas the control fibres had a marked gauge length effect, increasing by 45% on reducing the length from 5 to 1 cm. The Youngs moduli of the clean fibres and the controls were virtually the same after each heat treatment but this had been expected as both batches had been spun and processed in the same way. Scanning electron microscopy of the fracture surfaces of fibres heat treated to 1,400 and 2,500° C showed a complete absence of the recognisable internal flaws found in commercial carbon fibres and in all cases failure seemed to have started at the surface. The surface flaws in the control samples were more obvious than those in the clean fibre and an example of each is given in Fig. 2.

The results reported here emphasise the importance of removing impurities from the PAN precursor fibre and show that the decrease in strength with HTT previously found is not an inherent characteristic of carbon fibre. Recently Reynolds and Sharp[11] have put forward a failure mechanism based on shear taking place in misorientated crystallites and their model does predict strength to increase with HTT. Finally, it must be added that further increases in strength should be possible if the small flaws still present in the fibres could be removed. Not too much importance should be attached to the actual strength values of the carbon fibres quoted in this investigation but only trends should be considered. A total of 0.5 g was used. Much larger quantities give higher strengths, presumably due to some surface healing by carbon deposition from pyrolysis gases, as is well known. But it now seems obvious that the strength values of carbon fibres are a direct consequence of the external and internal cleanness of the initial PAN fibres.

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Precambrian Microfossils of Carpentarian Age from Bungle Bungle Dolomite of Western Australia

I WISH to present some preliminary results and interpretations of the examination of a well preserved assemblage of mid-Precambrian microfossils. This previously unknown microflora of blue-green and green or red algal affinities is from chertified stromatolitic dolomites of the Bungle Bungle Dolomite which outcrops in the Osmond Range of Western Australia. There are no published radiometric ages for this formation but the Mount John Shale Member of the overlying Wade Creek Sandstone has been dated (Rb/Sr method) at $1.128 \pm 0.1 \times 10^9$ yr BP[1]. Regional studies and the angular unconformity between the Bungle Bungle Dolomite and Wade Creek Sandstone indicate that the former is considerably older than this minimum and that an age of about 1.5×10^9 yr for the microbiota described here is consistent with the data available. The Bungle Bungle Dolomite consists of at least 1.3 km of dolomite, dolomitic shales, shales and sandstones. Well preserved stromatolites occur at certain levels and have been briefly described elsewhere[1]. The samples used in this investigation are from the measured section in the upper part of the formation at 17° 13′ S 128° 23′ E.

The microfossils are preserved in dark brown to black, fine grained cherts. Organic matter, either in a finely divided, unstructured form or as structurally preserved microfossils is responsible for the dark colour of the chert. In petrographic thin section (30 μm thick) the organic matter is pale amber to dark brown with the microfossils being generally darker than the intimately associated unstructured material. The chertified horizons in the Bungle Bungle Dolomite have a complex diagenetic history the elucidation of which is further complicated by the effects of tropical weathering. Dolomite and silica minerals are mutually replacive[2,3] making unequivocal interpretation of petrographic evidence difficult. In broad terms, field and petrographic data indicate that the fossiliferous chert is diagenetic in origin, often replacing the original carbonate sediment.

In some of the microfossils, fine structural details are preserved, while others are modified or destroyed by diagenetic and subsequent processes. Extreme variations in the state of preservation are often juxtaposed and associated with subtle textural differences in the chert. Compaction and chemical alteration of much of the organic matter occurred before the initial chertification while the replacement of chert by very late dolomite is responsible for the destruction of much fossiliferous material.

Only a selection of the diverse Bungle Bungle microbiota is presented here and a more systematic account will be published elsewhere. A fairly restricted range of vegetative structure is exhibited by the coccoid forms in the assemblage. As a result of irregularities in fossilisation and subsequent processes, morphological characteristics are subject to great variation. This morphological inconsistency is superimposed upon natural variation making the delineation of discrete morphotypes, though possible, often difficult and very speculative.

The most common coccoid forms are unicells, dyads and tetrads of cells 1–5 μm in diameter (Figs 1*a* and *b*) many of which possess double or multilayered walls. This broad group, which includes at least four types, may occur as isolated cells, in clusters of up to a hundred cells or in close, almost colonial associations. The cells of another group with similar gross morphology contain inclusions, which may be in the form of dark spots about 1 μm across (Fig. 1*c*) or as apparently membrane-bound structures 2–3 μm in diameter containing minute dark granules (Fig. 1*d*). Isolated colonies of spherical to ellipsoidal cells 2–4 μm in diameter are not uncommon (Fig. 1*e*). Individual colonies may be up to 50 μm across, comprising several hundreds of cells, many of which contain minute (about 0.5 μm) inclusions, and whose shape may be modified by mutual compression. Some of these colonies are preserved in the process of disintegration into smaller colonies or into separate cells.

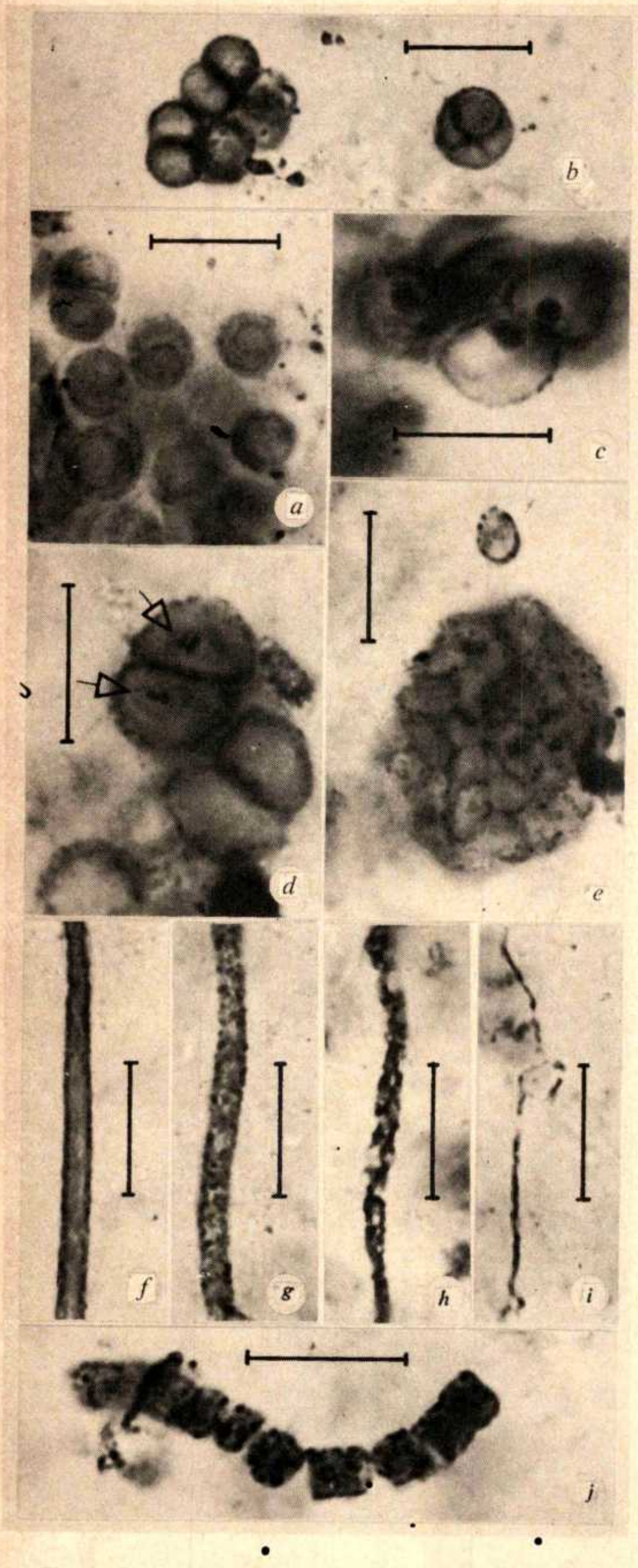

Fig. 1 Structurally preserved organic microfossils from the Bungle Bungle Dolomite. All micrographs are of petrographic thin sections of chertified stromatolites in plane polarised light. All scale bars represent 10 μm.

Filamentous forms are less abundant and not as well preserved as the coccoid forms. The most common type of filament (Fig. 1*f–h*) is non-septate, unbranched, 1.5–2 μm in diameter and up to 500 μm long. Variations in preservation often give these filaments a septate appearance (Fig. 1*g* and *h*). Smaller filaments 0.6 μm or less in diameter occur and variously seem to be septate, non-septate, spirally twisted or composed of strings of rod-shaped cells (Fig. 1*i*). It is difficult to determine whether these are separate types or preservational artefacts, since their detailed morphological features are at or below the limit of effective light optical resolution. Septate filaments are rare and usually fragmentary with the constituent cells often nearly separate. The most frequent type (Fig. 1*j*) is composed of cylindrical cells 2.5–3.5 μm in diameter and 1.5–2.5 μm long with apices terminated by a small hemispherical cell.

The size, vegetative form, and morphology of most of the microbiota are comparable with living cyanophycean algae. Coccoid forms with layered walls resemble members of the Chroococcaceae, and the septate filaments look like trichomes of the Oscillatoriaceae. Living oscillatoriaceans possess the facility of gliding motility and the non-septate filaments may be abandoned sheaths of such algae. The specimens arrowed in Fig. 1*d* resemble plasmolysed cells. Since the protoplasm of living blue-green algae is highly viscous and difficult to plasmolyse[4] it may be that, as suggested elsewhere[5], such forms (Fig. 1*c* and *d*) represent eukaryotic algae (Chlorophyceae or Rhodophyceae) with their contents and organelles preserved.

Stromatolites are laminated organosedimentary structures that at the present day are formed in shallow water by associations of algae dominated by the Cyanophyceae. The striking similarity between detailed sedimentological features and the morphologies of the associated organisms in Recent[6,7] Bungle Bungle, and other stromatolitic deposits[5] supports the interpretation of the Bungle Bungle microbiota as a stromatolite-producing assemblage of coccoid and filamentous cyanophycean algae associated with less common coccoid eukaryotic algae.

In an evolutionary context the Bungle Bungle microbiota is comparable with other Precambrian stromatolitic assemblages, especially that from the Amelia Dolomite[8], and is consistent with the evolutionary models proposed by Cloud[9] and Schopf[10,11]. It must be stressed, however, that stromatolitic microbiotas on which these models are largely based, are from the same restricted environment and hence, represent a strongly biased sample of Precambrian life. In the Bungle Bungle microbiota, however, elements from other environments are present and exemplified by the colonial forms (Fig. 1*e*) which are not members of the stromatolite-producing assemblage but invariably occur in sediment/detritus rich laminae. These allochthonous forms and microfossils described elsewhere[12,13] from non-stromatolitic Precambrian sediments suggest that life had become considerably diverse by 1.5×10^9 yr BP and that much remains to be discovered about the ecological diversifications and evolution of early life.

Dr M. D. Muir (Imperial College, London) and Mr K. A. Plumb (BMR, Canberra) collected the samples described here in 1972. I acknowledge their help and encouragement during the work which was sponsored by a research assistantship from the Natural Environmental Research Council.

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Spheroidal particles produced by cavitation erosion

SMOOTH spherical particles have been observed during the microscopic analysis of wear surfaces (ref. 1 and R. S. Miller, personal communication) and of wear particles[2] using a new technique called ferrogram developed by Westcott[2]. These investigators speculated that the spherical particles were produced by cavitation bubbles occurring at the contact surfaces of the bearing. The possibility of cavitation in bearings is well known[3]; however, it was difficult to understand how these bubbles could produce such perfect microspheres (in the range of 1 to 10 μm in diameter). To verify that cavitation erosion could indeed produce such spheres, we conducted careful experiments with the help of the American Society for Testing and Materials (ASTM) standard vibratory cavitation erosion apparatus; this communication reports the results of this investigation.

R. S. Miller (personal communication) noticed a spherical particle in one of the scanning electron micrographs (taken by Sperry Corporation) of an automotive ball bearing (Fig. 1*a*). About the same time, Scott and Mills[1] and Westcott[2] observed such spherical particles in bearings. Scott and Mills observed them on the fracture surfaces of a ball but Westcott used his new technique[2], called ferrographs, and observed them in various lubricating fluids after their use in bearings.

At the suggestion of Doroff and Miller, A.P.T. conducted controlled experiments using the ASTM standard vibratory cavitation erosion apparatus and ASTM standard test procedures. Tests were conducted on annealed SAE 52100 steel (supplied by the Timken Company) in SAE 10w non-detergent (Quaker State) lubricating oil and on aluminium 1100-F in distilled water.

Analysis of the lubricating oil samples done at Trans-Sonics by S.W.D., A.P.T. and V.C.W. using the ferrographic technique revealed the existence of spheroidal particles. Fig. 1*b* shows optically perfect spheres produced by cavitation of SAE 52100 steel in SAE 10w oil. Similar analysis of the oil samples and of the aluminium erosion particles using techniques developed at the Institute of Ocean Science and Engineering of Catholic University also revealed such spherical particles (Fig. 1*b*). Using an electron probe microanalyzer A.P.T. conducted X-ray analysis to determine the composition of these micro-spheres. Figure 1*c* and *d* show that the spheroids are indeed steel and aluminum.

This discovery that cavitation erosion can produce very smooth spheres has many practical and scientific implications in identifying, understanding, and solving many erosion and wear problems. We are pursuing these investigations further.

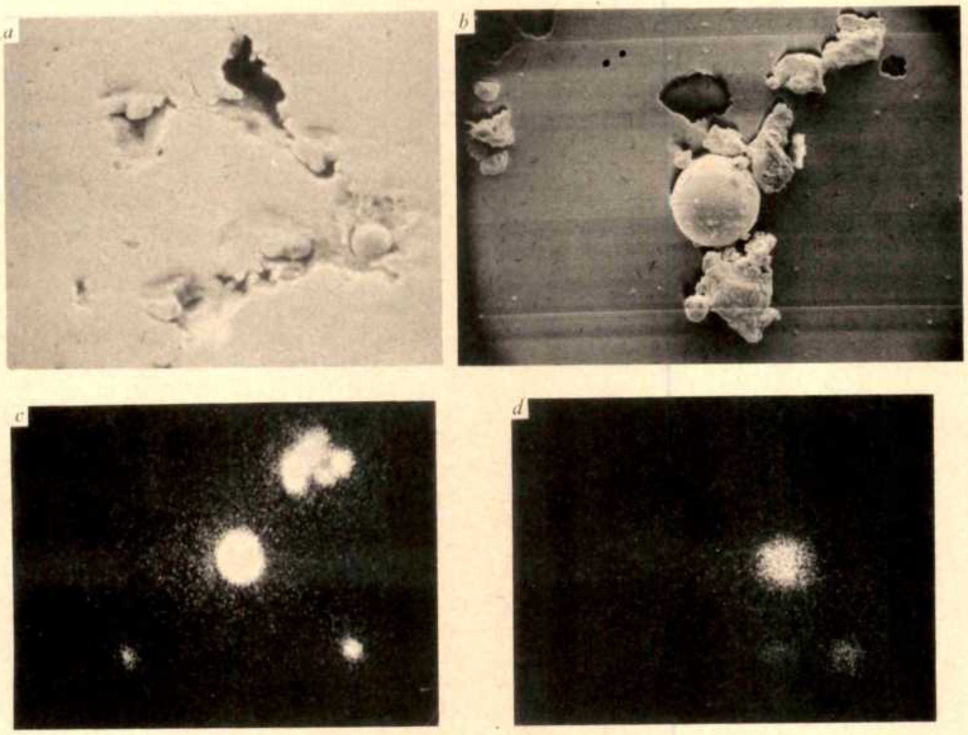

FIG. 1 *a*, Spherical particle observed in microcrack in an automotive ball bearing (R. S. Miller, personal communication); *b*, photograph of a smooth spherical particle observed in the oil cavitated for 40 h in an ASTM standard cavitation erosion apparatus (courtesy of Dr Ruff at the National Bureau of Standards); *c*, X-ray image scanned for aluminium in an electron probe microanalyser; *d*, X-ray image scanned for steel in an electron probe microanalyser.

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Nephrite (jade) Occurrence in the Great Serpentine Belt of New South Wales, Australia

THIS communication is to report the presence in the Great Serpentine Belt of New South Wales, Australia[1–3] of a nephrite (jade) occurrence. Within the Great Serpentine Belt that outcrops discontinuously for more than 350 km in northern New South Wales[4] (Fig. 1) several economic mineral occurrences have been discovered and developed in recent years. The Woodsreef asbestos deposit, near Barraba, the largest deposit of its type in the Southern Hemisphere, is by far the best known of the recent discoveries.

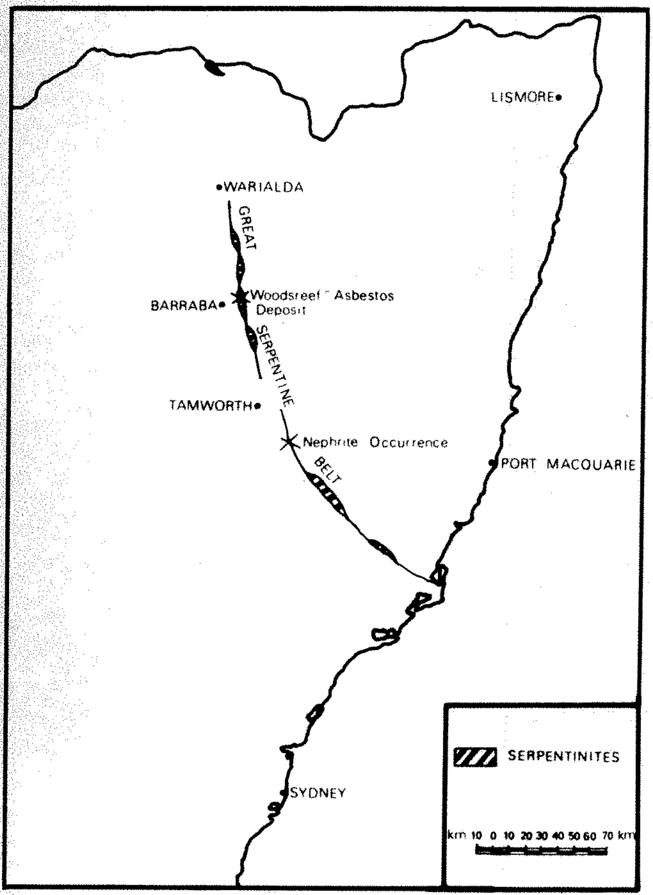

FIG. 1 Location of the Great Serpentine Belt in nothern New South Wales, Australia.

The lensoidally shaped nephrite occurrence 24 km south-east of Tamworth may prove to be Australia's first economic deposit as high quality gemstones have been cut from the occurrence. Geological investigations are currently in progress to determine the size, overall grade and economic potential of this very interesting mineral occurrence.

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BIOLOGICAL SCIENCES

Transamination is a Major Pathway of L-Dopa Metabolism following Peripheral Decarboxylase Inhibition

WHEN L-3,4-dihydroxyphenylalanine (L-dopa) is administered to human subjects[1], the greater proportion is metabolised by decarboxylation, a small amount by O-methylation and traces only by transamination[2]. It seems likely that the benefit resulting from L-dopa therapy in Parkinsonian patients derives largely from the generation of one of its metabolites, dopamine, by decarboxylation within the central nervous system[3]. Less than 5% of administered L-dopa becomes available for this purpose: most of the dose is decarboxylated peripherally[4]. There is good reason to believe that the dopamine so formed is unable to penetrate the blood-brain barrier to any great extent[5], so that little of the original dose is available to contribute to the therapeutic response. By blocking peripheral decarboxylase, however, certain decarboxylase inhibitors[6,7], which are themselves unable to cross the blood-brain barrier to any significant extent[8], bring about an accumulation in the plasma of administered L-dopa, thus providing a higher concentration gradient to enter the central nervous system and allowing lower oral doses of L-dopa to be employed therapeutically[9].

It has so far been assumed that, apart from some increase in the formation of 3-O-methyldopa[10], peripheral degradation of L-dopa is largely prevented by the concomitant use of a decarboxylase blocking drug. We now present evidence that the combination of drugs results in a major diversion of L-dopa metabolism to a pathway where transamination substitutes for decarboxylation.

Thirteen patients with idiopathic Parkinsonism, taking part in a double blind comparison of L-dopa plus L-α-methyl-dopahydrazine (MK 486) against L-dopa alone[11], were investigated. Urine samples (24 h), preserved at −15° C with 6 N HCl (25 ml), were collected during the following treatment regimens: (1) before treatment (controls); (2) whilst receiving an optimal oral dose of L-dopa (1.0–5.75 g per 24 h; mean=3.5 g per 24 h) ('normal' dose experiment); and (3) whilst receiving a reduced dose of L-dopa (0.1–2.0 g per 24 h; mean=0.65 g per 24 h) plus oral MK 486 (300 mg per 24 h) ('low' dose L-dopa plus MK 486 experiment). Urine samples were also collected from (4) three of these patients during treatment with their own particular reduced dose of L-dopa (0.3–1.1 g per 24 h; mean 0.62 g per 24 h) employed during regime (3) but in the absence of MK 486 ('low' dose L-dopa experiment). Urinary *m*-hydroxyphenylacetic acid (*m*-HPAA), *p*-hydroxyphenylacetic acid (*p*-HPAA), homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were quantified as described previously[12,13], except that silylation was performed at 65° C for 1 h, using N,O-*bis* (trimethylsilyl)-acetamide (BSA, 0.2 ml) and dioxane (0.1 ml) containing *n*-eicosane (8 μg ml^{-1}) as internal standard. Urinary 4-hydroxy-3-methoxyphenyllactic acid (VLA), 4-hydroxy-3-methoxyphenylglycol (HMPG) and 4-hydroxy-3-

Table 1 Effect of MK 486 on urinary metabolites of L-dopa

Drug regimen	*m*-HPAA	*p*-HPAA	HVA	Metabolite DOPAC	VLA	HMPE	HMPG
Control experiment ($n=13$)	3.4 (0.52)	9.6 (0.85)	6.6 (0.60)	2.8 (0.34)	0.1	0.17 (0.01)	1.64 (0.12)
'Normal' dose experiment on L-dopa alone ($n=13$)	10.0 (1.73)	11.2 (1.36)	801 (169)	589 (132)	16.0 (4.9)	1.76 (0.37)	2.19 (0.36)
'Low' dose experiment on L-dopa alone ($n=3$)	5.6 (0.92)	13.3 (3.01)	142† (39.1)	91.3† (39.8)	2.6* (0.86)	0.39 (0.20)	1.55 (0.28)
'Low' dose experiment on L-dopa plus MK 486 ($n=13$)	5.7 (1.29)	13.5 (2.59)	52.5† (11.6)	17.0† (4.36)	96.5* (19.1)	0.30 (0.03)	1.74 (0.25)

m- and *p*-HPAA = *m*- and *p*-hydroxyphenylacetic acid; HVA = homovanillic acid; DOPAC = 3,4-dihydroxyphenylacetic acid; VLA = 4-hydroxy-3-methoxyphenyllactic acid; HMPE = 4-hydroxy-3-methoxyphenylethanol; HMPG = 4-hydroxy-3-methoxyphenylglycol.

Values are means ± s.e.m. (bracketed), expressed as mg per 24 h.

Significance of difference between respective urinary metabolites in patients on 'low' dose alone with those on 'low' dose plus MK 486. * = $P<0.05$. † = $P<0.01$.

methoxyphenylethanol (HMPE) were assayed as before[14], except that the derivatised extracts were finally reconstituted in ethyl acetate (0.3 ml) containing α-1,2,3,4,5,6-hexachlorocyclohexane (α-HCH, 1 μg ml^{-1}) as internal standard.

The results are given in Table 1. In reduced dose experiments, the urinary excretion of *m*- and *p*-HPAA, HMPG and HMPE was relatively unchanged by MK 486. There was, however, a highly significant drop in output of the decarboxylation pathway metabolites, HVA and DOPAC, during MK 486 treatment, in agreement with the findings of others[15]. In accordance with a prediction by one of us[2], there was a large increase in output of the transamination metabolite, VLA. The extent of this shunting to an alternative degradation route is demonstrated in Fig. 1 where values are shown for three patients who were, respectively, receiving 300, 1,100 and 400 mg L-dopa per 24 h, with and without MK 486. A complete reversal in the ratios of decarboxylation to transamination series metabolites was observed, a useful index in itself, perhaps, of *in vivo* decarboxylase inhibition.

To what extent the increased output of VLA represents transamination of L-dopa itself followed by O-methylation rather than degradation of 3-O-methyldopa (Fig. 2) cannot be decided on the evidence so far available. The amount of 3,4-dihydroxyphenyllactic acid present might have been expected to shed light on the problem. It was indeed sought but not detected in any sample, even though reference standards taken through the procedure were recovered from urine in high yield. This finding was first interpreted as indicating that the VLA derived solely from 3-O-methyldopa, of which it is known to be the major metabolite[16]. A study of the further metabolism of immediate keto-acid transamination products in the rat (B. L. Goodwin, C. R. J. Ruthven and M. Sandler, in preparation) has, however, revealed the rather surprising finding that 4-hydroxy-3-methoxyphenylpyruvic acid is freely converted to the corresponding lactic acid whereas 3,4-dihydroxyphenylpyruvic acid does not undergo this type of reduction. The solution to this problem must await further study of the phenyllactate-forming enzyme[17,18] and quantitative analysis of the pyruvic acids themselves, a procedure which has not so far been technically feasible because of the formation of multiple derivatives.

With high concentrations of L-dopa in the circulation protected from decarboxylation, there seems to be no very good reason why considerable amounts of 3,4-dihydroxyphenylpyruvic acid should not be generated. In terms of the therapeutic response, the role of any Schiff base which might

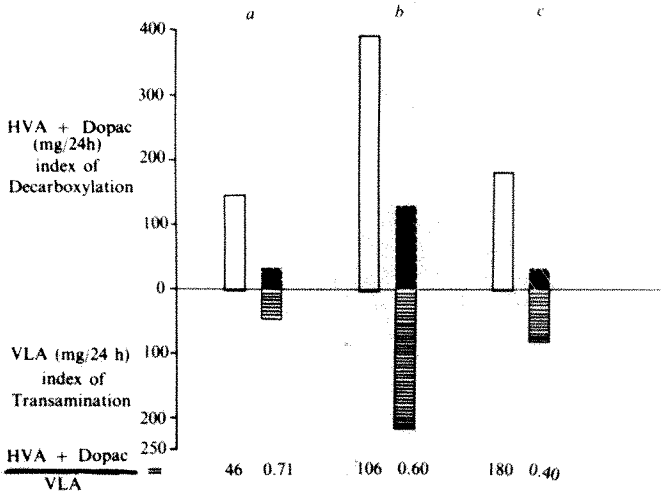

Fig. 1 24 h urinary output of 4-hydroxy-3-methoxyphenyllactic acid (vanillactic acid, VLA) before (amounts too small to illustrate clearly) and after (horizontally shaded columns) and homovanillic acid (HVA) plus 3,4-dihydroxyphenylacetic acid (DOPAC) before (open columns) and after (diagonally striped columns) peripheral decarboxylase inhibition with MK 486 in three Parkinsonians taking oral L-dopa as follows: Patient $a=$ 300 mg, $b=$ 1,100 mg, $c=$ 400 mg per 24 h.

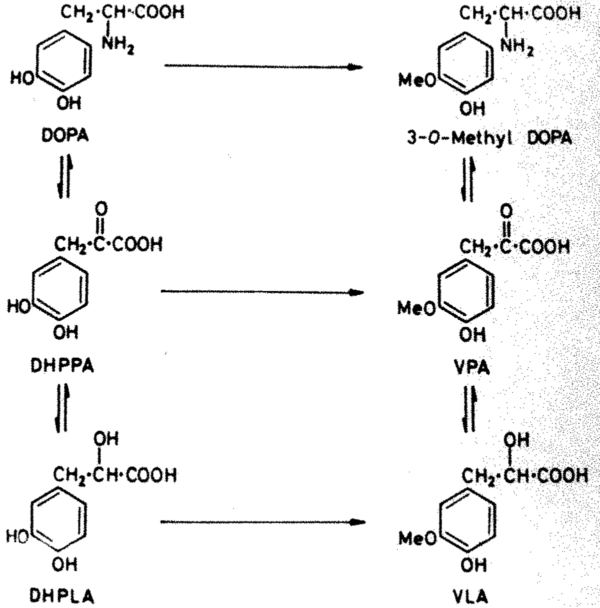

Fig. 2 Transamination pathways of L-dopa metabolism.

be derived from it[19] is still speculative. As predicted[12], this keto-acid is a good substrate for *p*-hydroxyphenylpyruvate oxidase[20], which converts it to 2,4,5-(3,4,6-)trihydroxyphenylacetic acid. The trihydroxy acid has recently been identified unequivocally in the urine of Parkinsonian patients during L-dopa treatment (J. H. Fellman, personal communication). Its distribution of ring hydroxy groups in relation to the side chain is identical to that of 6-hydroxydopamine, the effects of which on the central or peripheral nervous system can be dire[21] once it is taken up into catecholamine-binding sites. It is to be hoped that concentrating mechanisms do not exist for the trihydroxy acid which could well possess similar properties if present in high enough local concentration.

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Evidence from *Tfm*/0 that Androgen is Inessential for Reproduction in Female Mice

THE testicular feminisation syndrome in affected XY male mice is the result of the action of the X-linked *Tfm* gene[1], which causes a failure of target organs to respond to androgen. The question arises whether genetic females affected with the gene would be reproductively abnormal in any way. Androgen is produced in the normal mouse ovary; its functional significance is not understood but it has been claimed that a response to androgen is essential for ovulation and mating[2]. If so, then females homozygous or hemizygous for the *Tfm* gene (*Tfm*/*Tfm* or *Tfm*/0) might be unable to reproduce. Ohno *et al.*[3], have recently described six *Tfm*/0 female mice which collectively showed remarkably rapid ovarian degeneration, more rapid than in +/0 mice, thus leading to the conclusion that a response to androgen is necessary for normal ovarian function in mice. The fertility of these mice had not, however, been tested. We here report results with two *Tfm*/0 mice, both of which proved to be fertile and produced litters.

Both animals were born in an experiment in which female mice were irradiated with a dose of 100 rad X rays on the morning after conception, a treatment which yields a high frequency of XO offspring[4]. The parental crosses were *Tfm* + +/+ *Ta*+ ♀× + + *Gs*/Y ♂, and the two *Tfm*/0 animals were the progeny of different pairs several months apart. Both were wild type in colour and externally looked female. They were distinguished from *Tfm*/Y by the fact that their vaginae opened at an age typical for normal females, that is, before 6 weeks, whereas in *Tfm*/Y the vagina typically does not open until about 3 months of age.

The two animals were mated at 46 and 39-d-old to a *Blo*/Y and a *Ta*/Y male respectively. Both proved fertile, the first having four pregnancies and the second five (Table 1). The offspring confirmed the presumed genotypes of the mothers: there were typical *Tfm*/Y offspring, confirming that both mothers carried the *Tfm* gene, and presumed XO offspring, indicating that both mothers were themselves XO. This latter point was also confirmed by chromosome counts, on tissue culture of ear skin for the first and on corneal epithelium for the second animal. In both cases these showed only thirty-nine chromosomes. Therefore, both mice were genetically proven to be *Tfm*/0.

Since these animals obviously ovulated and mated repeatedly, went through normal pregnancies, and reared offspring, it is clear that, in the mouse at least, a response to androgen is not essential for any of these functions.

The question arises whether the continued fertility up to 6 months of age of our two animals is in conflict with the

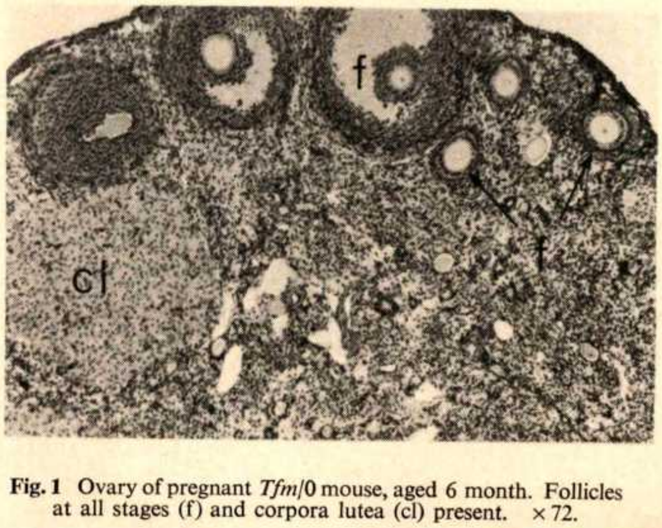

Fig. 1 Ovary of pregnant *Tfm*/0 mouse, aged 6 month. Follicles at all stages (f) and corpora lutea (cl) present. ×72.

finding[3] of Ohno *et al.* of marked deterioration in *Tfm*/0 ovaries as early as 8 weeks. The reproductive performance, in terms of litter size, number of litters, and reproductive lifespan of our animals was within the range found for XO mice[5], but below average. Thus on the evidence of reproductive performance their ovaries might have been either more severely affected than +/0 ovaries, or comparable with them. Histologically, however, our findings did differ from those of Ohno *et al.* In serial sections the ovaries of both animals contained apparently viable oocytes and were no more severely affected than those of +/0 animals of comparable age. In the second animal, killed at 6 months of age, the only obvious abnormalities were a shortage of primordial oocytes and an excess of atretic follicles (Fig. 1). Otherwise the ovary was essentially normal, with follicles at all stages and a number of corpora lutea. Many of the antral follicles were degenerating, but this animal was pregnant when killed and degeneration of large follicles is characteristic of pregnancy[6].

Table 1 Breeding tests of two *Tfm*/0 mice

Animal			Age (d)	No. born	No. weaned	Offspring		
1	Mated		46			*Blo*/+	*Blo*/*O*	*Tfm*/Y
	Litter	1	70	3	3	3	—	—
		2	90	5	5	3	—	2
		3	132	2	2	2	—	—
		4	175	2	2	1	1	—
	Killed		277					
						Ta/+	*Ta*/*O*	*Tfm*/Y
2	Mated		39					
	Litter	1	63	2	—	—	—	1
		2	95	6	6	2	2	2
		3	131	1*	—	—	—	—
		4	159	1*	—	—	—	—
		5	182	—†	—	—	—	—
	Killed		182					

All the offspring were externally female. The *Tfm*/Y offspring were classified as such by the finding of testes on dissection. There is no reason to link the second animal's poor rearing ability with *Tfm*/0 condition, since normal mice often fail to rear litters of only one or two young, through insufficient stimulus to lactation.

* Pregnancy diagnosed by palpation; one foetus, but no live young seen. Foetus presumed stillborn.

† Pregnant when dissected; three dead implants only.

The ovaries of the older *Tfm*/0, killed at 9 months of age, were more abnormal (Fig. 2). The number of oocytes at all stages was markedly reduced, whereas that of corpora lutea was much increased, so that the bulk of the ovary consisted mainly of corpora lutea. But the normal ovarian architecture still remained and there was no sign of tumour or cyst formation. Such oocytes as there were appeared to be developing normally, up to stage 8 or 9 (ref. 7). The general picture was very similar to that found by Lyon and Hawker[5] in a 9-month-old +/0 animal, and apparently was less severe than that found by Ohno *et al.*, in a *Tfm*/0 of only 112 d. Thus, from our animals there is no evidence that a response to androgen is required for normal ovarian function.

The reasons for the discrepancy between Ohno's results and ours are not clear. Possible explanations include the following.

(1) Our animals were both with a male continuously from about 6 weeks of age. Therefore the ovaries will have been under the hormonal influence of corpora lutea of pregnancy, pseudopregnancy or lactation for most of the time. Ohno *et al.* suggested that perhaps this would tend to maintain ovarian normality. Pregnancy does not, however, affect the oocyte count in normal animals, although it does tend to prevent abnormalities of ovarian structure[8]. In order to explain the effects of *Tfm*/0 mice on this basis therefore one might need to postulate that pregnancy protected the ovaries more strongly in them than in normal mice.

(2) Ohno *et al.*[9] have found that the expression of the *Tfm* gene in the male can be altered by genetic factors on the X chromosome apparently similar to the controlling elements found by Cattanach[10]. Possibly these elements also affect expression in females, and Ohno's stock and ours have come to differ in some unknown way in respect of them.

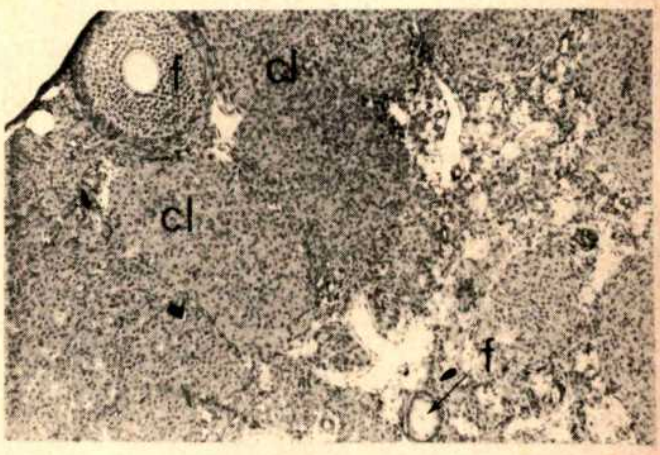

Fig. 2 Ovary of *Tfm*/0 mouse, aged 9 month. Very few follicles (f), but numerous corpora lutea (cl). Both fixed in Heidenhain's Susa, stained haematoxylin and eosin. ×72.

(3) Perhaps the general genetic background of the stock affected the expression of *Tfm*/0 and +/0 in the ovaries. The numbers of oocytes, the age when all oocytes are depleted, and also changes with age in reproduction and ovarian structure vary markedly with strain in normal animals[8] and so it would not be surprising if they also varied in XO mice.

It could, however, be that none of these explanations is correct, and until more is known there must remain some doubt concerning the importance of androgen in reproduction of female mice. We have shown that androgen is inessential for ovulation, mating, pregnancy and lactation. The results of Ohno *et al.* suggest that it is essential to maintain normal ovarian function; our own raise uncertainties about this point.

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Role of Hydrogen Bonding in Organic Cation Discrimination by Gallbladder Epithelium

THE main route of ion permeation across some epithelia, including that of the gallbladder, is by the so-called 'tight' junctions between adjacent cells[1]. In the case of the gallbladder, this pathway is much more permeable to cations than to anions, and it also discriminates markedly among different inorganic cations[2,3]. We have extended selective permeability measurements to a series of small amines in the form with one positive charge (compare ref. 4). The rationale behind these experiments is that permeability differences among cationic amines may help to identify the chemical nature and arrangement of ion-selective sites, as well as the possible existence of steric restraints in the permeation channel. The results indicate that gallbladder 'tight' junctions contain proton-acceptor sites which distinguish more markedly among cationic proton donors than does water.

Cation permeability ratios (P_x/P_{Na}) were measured as described previously[2,3,5], in flat sheets of bullfrog gallbladder mounted between two well-stirred chambers at 23° C. Briefly, transepithelial electrical potential differences (dilution potentials and biionic potentials) were measured with AgCl electrodes, and P_x/P_{Na} values were calculated by inserting these potentials into a Goldman–Hodgkin–Katz equation modified for the presence of a shunt[3]. For all amines studied, P_x/P_{Na} and gallbladder conductance were essentially constant throughout an experiment. Eighteen of the nineteen amines tested had pK_a values ≥ 9 and were studied at pH 7, while hydrazine (pK_a 8.1) was studied at pH 5. Thus, more than 99% of the dissolved amine was in the charged form. For amines with $pK_a \geq 9$, P_x/P_{Na} values measured at pH 5 and at pH 7 were found to be the same within experimental error.

Figure 1 displays the experimental values of P_x/P_{Na}, plotted as a function of the free-solution mobility ratios u_x/u_{Na}. For the nineteen amines as a group, it is obvious that P_x/P_{Na} neither equals, nor is a monotonic function of, u_x/u_{Na}. P_x/P_{Na} values are not even approximately in the sequence of reciprocal ionic sizes or of pK_a values. Figure 1, however, does exhibit two simple patterns. First, if one compares solutes with similar u_x/u_{Na} values, P_x/P_{Na} increases with the number of protons (≤ 1, 2, 3, or ≥ 4) that the solute has available for hydrogen bonding with a suitable proton acceptor. For example, among amines with u_x/u_{Na} in the range of 0.65 to 0.80, the P_x/P_{Na} sequence is 0.55 for $H_2N^+{=}C(NH_2)CH_3$ (four donor protons); 0.35 for $H_3N^+{-}CH_2{-}CH{=}CH_2$ and 0.33 for $H_3N^+{-}CH_2{-}CH_2{-}CH_3$ (both with three protons); 0.18 for $H_3C{-}NH_2^+{-}CH_2{-}CH_3$ (two protons); and 0.02 or less for $(H_3C)_4N^+$ (no proton). The second pattern is that, among amines with a given number of donor protons (≤ 1, 2, 3, or ≥ 4), P_x/P_{Na} values fall in the same or approximately the same sequence as u_x/u_{Na} values. For solutes with ≤ 1 proton, P_x/P_{Na} is too low to measure (that is, ≤ 0.02), regardless of the u_x/u_{Na} value.

Thus, there are four 'levels' of permeability, depending on the solute's available number of donor protons. Within each 'level', differences in molecular size or shape generally affect gallbladder P_x/P_{Na} values and free-solution u_x/u_{Na} values in the same sequence.

This relation between permeability and hydrogen-bonding ability indicates that gallbladder 'tight' junctions contain proton-acceptor sites, which distinguish more markedly among cationic proton donors than does water. Let us represent a solute's hydration energy as ΔF_{hyd}; its attraction energy to membrane sites (mainly due to hydrogen-bond forces, which include electrostatic forces), as ΔF_{mem}; the free-energy change in transferring solute from water to membrane sites, as ΔF_{trans}; and the solute's partition coefficient between membrane sites and water, as k. Then $\Delta F_{trans} = \Delta F_{mem} - \Delta F_{hyd} = -RT\ln(k)$. If, for two solutes a and b, b having more donor protons than a, the difference between the bonding energies in the membrane is greater than the difference in water (that is, $\Delta F_{mem,b} - \Delta F_{mem,a}$ more negative than $\Delta F_{hyd,b} - \Delta F_{hyd,a}$), then the membrane/water partition coefficient k will be larger for the solute b with more donor protons. Assuming (in analogy with other systems[6,7]) that increased solute/membrane forces will increase the solute's partition coefficient by a factor larger than the factor by which they reduce the solute's mobility in the membrane, the net effect would be that the solute with more donor protons will have the larger permeability. The absence of higher permeability 'levels' than the level associated with four donor protons suggests that ΔF_{trans} is not increased by addition of further donor protons beyond four. Comparison of enthalpies of solution for alkylammonium ions with varying numbers of donor protons, in the solvents water, dimethyl sulphoxide, and propylene carbonate[8], indicates that a dimethyl sulphoxide or propylene carbonate 'membrane' would resemble the gallbladder in its selective permeability to these cations. Note that membrane ligands need not necessarily form stronger H-bonds than water (ΔF_{mem} more negative than ΔF_{hyd}) to yield the pattern of Fig. 1; the ligands need only discriminate more among H-bond donors than does water ($\Delta F_{mem,b} - \Delta F_{mem,a}$ more negative than $\Delta F_{hyd,b} - \Delta F_{hyd,a}$).

The sodium channel of myelinated nerve[4], and lipid bilayers doped with any one of four carriers[9] (nonactin, trinactin, valinomycin, perhydroantamanide), resemble gallbladder in that selective permeability patterns exist for cationic amines

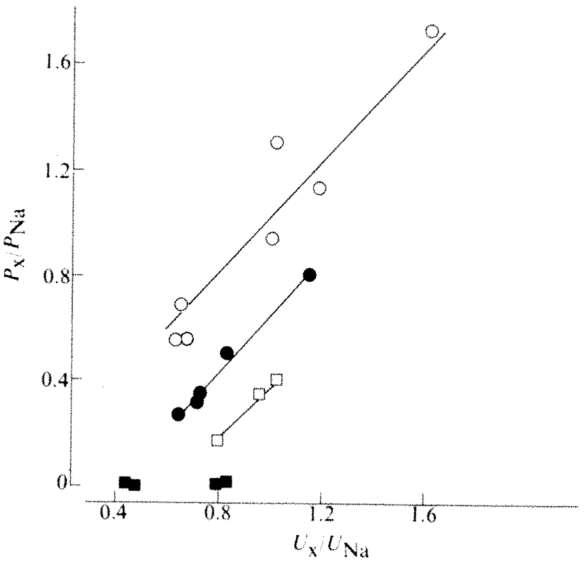
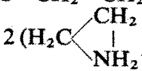


Fig. 1 Relative permeability coefficients in bullfrog gallbladder (P_x/P_{Na}, ordinate), as a function of relative free-solution mobilities (u_x/u_{Na}), for nineteen cationic amines in the form with one positive charge. P or u for a given cation (x^+) is expressed relative to P or u for Na^+. Each point represents the average value of P_x/P_{Na} for three to eight gallbladders. The number of donor protons that each cation has available for hydrogen-bond formation is, ○, ≥ 4 (NH_4^+, $H_2N^+{=}CH{-}NH_2$, $H_2N{-}NH_3^+$, $(H_2N)_2C{=}NH_2^+$, $H_3C{-}NH(NH_2)C{=}NH_2^+$, $H_2N{-}NH^+{=}C(NH{-}NH_2)_2$, $H_3C(NH_2)C{=}NH_2^+$); ●, 3 ($H_3C{-}NH_3^+$, $H_3C{-}CH_2{-}NH_3^+$, $H_2C{=}CH{-}CH_2{-}NH_3^+$, $H_3C{-}CH_2{-}CH_2{-}NH_3^+$, $(H_3C)_2CH{-}NH_3^+$); □, 2 ( $\mathrm{H_2C}\begin{smallmatrix}\mathrm{CH_2}\\ |\\ \mathrm{NH_2^+}\end{smallmatrix}$, $(H_3C)_2NH_2^+$, $H_3C{-}NH_2^+{-}CH_2{-}CH_3$); ■, 1 or zero, ($(H_3C)_3NH^+$, $(H_3C)_4N^+$, $(C_2H_5)_3NH^+$, $(C_2H_5)_4N^+$). The straight lines are regression lines fitted through each set of points, without attributing theoretical significance to the fit. u_x/u_{Na} values were calculated from measured conductances of 150 mM amine chloride (x^+Cl^-) solutions, assuming Λ_{Cl^-} to be the same as in a 150 mM KCl solution.

and are related to their H-bonding ability. Of the amines tested in these five systems as well as in gallbladder, the most permeant species are the proton-rich NH_4^+, $H_2N{-}NH_3^+$, $H_2N{-}HC{=}NH_2^+$, and $(H_2N)_2C{=}NH_2^+$ in all six cases. (The permeability sequence of these proton-rich amines differs, however, among the six systems, due probably to small differences in factors such as the strength of site/solute interactions. The close agreement in sequence, within a given proton 'level', between P_x/P_N for gallbladder and u_x/u_{Na} may be coincidental.) For the carriers nonactin and valinomycin, as for gallbladder, it was shown[9] by comparison of alkyl-substituted ammonium derivatives that removal of each successive proton of NH_4^+ reduces permeability. The main difference between the results for gallbladder and the other five systems is quantitative: the range of selectivity is much narrower in gallbladder. Thus, the relative permeability coefficients for NH_4^+, $H_3C{-}NH_3^+$, $(H_3C)_2NH_2^+$, and $(H_3C)_3NH^+$, respectively, are 1.00, 0.47, 0.20, ≤ 0.01 in gallbladder; 1.00, 0.0088, 0.00075, 0.000125 in valinomycin-doped bilayers; and 1.00, 0.00013, 0.00001, 0.000004 in nonactin-doped bilayers. By analogy with similar results in model systems[10], this finding indicates that the tight junctions of gallbladder provide a more hydrated environment than do carriers or the Na^+ channel of nerve, as was also concluded from analysis of alkali cation selectivity[1-3].

Figure 1 also illustrates how easy it is to be misled about the permeability pattern if only a restricted group of solutes is studied. For example, if only amines with the same number of donor protons are compared, the parallel between permeability ratios and free-solution mobility ratios would suggest that gallbladder is completely non-selective. Similarly, comparison only of alkylammonium ions such as NH_4^+, $H_3C{-}NH_3^+$, $(H_3C)_2NH_2^+$, and $(H_3C)_4N^+$ might suggest that selectivity results from molecular sieving in a narrow channel. In fact, an effective pore radius cannot be estimated simply from the size of the smallest impermeant amine, as permeability depends upon H-bonds as well as on size. Thus, $(H_3C)_4N^+$ is virtually impermeant, while $H_2N{-}NH^+{=}C(NH{-}NH_2)_2$, which is twice as large (but has many more donor protons), is quite permeant ($P_x/P_{Na}=0.55$). Again, $(H_2N)_2C{=}NH_2^+$, $(H_3C)_2CH{-}NH_3^+$, and $(H_3C)_2NH^+{-}CH_3$ have approximately the same size and shape, but the respective P_x/P_{Na} values are 0.94, 0.27, and ≤ 0.02, in the sequence of number of protons (six, three and one, respectively). To be able to detect any steric restriction, one must first recognise the pattern arising from H-bonds in the absence of steric effects. Differences between the bullfrog gallbladder results reported here and our unpublished permeability determinations for the same cationic amines in rabbit gallbladder seem to be due mainly to more marked steric restriction in the rabbit.

Finally, it is interesting to note that gallbladder permeability coefficients of amines in the uncharged form, and of most other non-electrolytes, decrease with increasing H-bonding ability[7,11]. Lipid-soluble non-electrolytes presumably cross gallbladder epithelium through the cell membranes rather than through the tight junctions, and a similar inverse relation between permeability and H-bonding ability is observed for membranes of single cells and for lipid bilayers. Such a pattern is exactly the opposite of the pattern described here for amines in the charged form permeating by tight junctions, where permeability coefficients increase with increasing H-bonding ability. Evidently the selectivity of tight junctions arises from the properties of their H-bonding sites, whereas the selectivity of cell membranes arises from the paucity of H-bonding sites in the bilayer interior compared with water[7,11]. Permeability studies with organic cations may prove similarly useful for characterising the molecular environment in other pore-like permeation pathways in biological systems.

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Intra- and Interpopulation Selection concerning the Alcohol Dehydrogenase Locus in *Drosophila melanogaster*

Lewontin and Hubby[1] have shown, by means of protein electrophoresis, that about one third of the loci in *Drosophila pseudoobscura* populations are polymorphic. Comparable studies by others have provided similar figures for a variety of species including man[2,3]. Some authors[4-6] have argued that the observed protein variation is merely a product of mutation and drift of neutral variants and is not maintained by selection. This view is severely criticised by others[7,8], who stress the importance of selective forces. Indications for the operation of selective forces have mainly come, until now, from studies on geographical variation[9,10]. Direct experimental evidence, concerning the relative selective values of electrophoretic variants is, however, scarce. In one well-documented case, Yamazaki[11] did not find selective differences between esterase-5 variants in *D. pseudoobscura*.

To obtain more relevant information on the nature of protein variation we studied the widespread polymorphism of the alcohol dehydrogenase locus in *D. melanogaster*. The *Adh*-locus is located on the second chromosome (2–50.1) and two alleles (Adh^S and Adh^F) are known from natural populations[12]. To characterise *Adh*-genotypes agar gel electrophoresis was carried out on whole fly homogenates for 1 h at 140 V and 28 mA in 0.02 M veronal buffer, *p*H 8.5. After electrophoresis the gels were transferred to a solution consisting of 97 ml 0.1 M veronal buffer *p*H 8.5, 3 ml ethanol 96% and 50 mg NAD. After 15 min the sites of alcohol dehydrogenase activity on the gel can be distinguished by fluorescence under ultraviolet light (366 nm). We applied the nomenclature of Grell[12] for the description of the slow and fast alleles. Five homozygous S-lines and five homozygous F-lines were isolated from the polymorphic Groningen population by making single pair matings. Each homozygous line originated from a mating in which both parents were homozygous for the same allele. The Groningen base population was founded three years before with several inseminated females collected at a local fruit market and since kept in the laboratory as a cage population with several thousands of individuals. The frequency of the F-allele fluctuated between 0.50–0.65 during that time.

To investigate whether selection occurs, cage populations

Table 1 Change in frequencies of the *Adh*^{F}-allele in cage populations (based on samples of seventy-five ♀♀ and seventy-five ♂♂)

Initial frequency	Frequency after 5 months	Frequency after 19 months
0.20	0.37	0.66
0.20	0.55	0.54
0.40	0.55	0.59
0.40	0.54	0.63
0.60	0.55	0.45
0.60	0.65	0.64
0.80	0.60	0.60
0.80	0.64	0.68
0.62*	0.53	0.54

* Groningen base population.

were started with different allelic frequencies (0.20, 0.40, 0.60 and 0.80 F). The rationale of this experiment is that in the case of neutral alleles, the frequencies will show small fluctuations around the initial values, and should differential selection work, there will be determinate changes. Each population was started with 200 pairs of flies from the homozygous S- and F-lines in the appropriate proportions; each initial frequency was represented by two cages. Allelic frequencies were determined twice: 5 and 19 months after the start of the experiment. For this purpose, fresh food cups were inserted in the cages for one day, and from the flies emerging from these cups, a sample of seventy-five ♀♀ and seventy-five ♂♂ was electrophoresed for genotype identification. Marked changes in allelic frequencies occurred after 5 months (approximately ten generations) (Table 1). An increase is observed in the 0.20 and 0.40 F populations, a decrease is found in the 0.80 F population, as in the 0.60 F populations no distinct changes occur. A second sample of flies, taken from the cages after 19 months (approximately thirty-eight generations), shows allelic frequencies lying between 0.45–0.68 F.

Another experiment was designed to measure the survival of populations, differing in genetic composition with regard to the *Adh*-locus, in more or less extreme environments. For this purpose four kinds of populations were started in vials using flies from the homozygous S- and F-lines: monomorphic F; monomorphic S; polymorphic with a frequency of 0.20 F; and polymorphic with a frequency of 0.80 F. Each of these four population types was represented with 50 vial populations in each of the nine environments listed in Table 2. Each vial population was started with ten females, mated previously with the appropriate males. The following procedure was then applied: the parents were removed and discarded before the emergence of their offspring, all flies from the new generation were shaken into vials with fresh food, without mixing between vials. This was repeated each generation, the time of transfer varying between environments because of the differences in development time. Extinction is defined as the proportion of vials which fail to produce at least one pair of offspring in a given generation.

The outcome of the extinction experiments is given in Table 2, where cumulative extinction percentages after eight generations are listed. It seems that the severity of the environmental stress differs considerably among environments. Table 2 also shows that, though in some environments there are no differences in survival, population types differ greatly in extinction frequency in others. A test of independence shows that extinction frequency is not independent of population type in environments 5, 6, 7, 8 and 9. The monomorphic S population type shows, relative to the other population types, the highest extinction rate on ethanol-supplemented medium and at low temperature. This population type shows on the contrary, the lowest extinction rate in high humidity conditions, where the monomorphic F type has the highest extinction rate. At high temperature, both polymorphic population types have a lower extinction rate compared with the monomorphic ones, the opposite is true in low humidity conditions.

In addition to measuring extinction, allelic frequencies were determined in both types of polymorphic population types in environments 1, 2, 4, 5 and 6. For this purpose, a variable number of flies derived from ten randomly chosen vials were electrophoresed (Table 3). In all but one of the investigated environments identical allelic frequency changes occur: the 0.20 F populations show an increase to values >0.40 F, the 0.80 F populations a decrease to values between 0.60 and 0.70 F. The one exception is the ethanol-supplemented medium, in which the 0.20 F populations show a much more pronounced rise and the 0.80 F populations also show an increase.

What do these findings mean for the selection *versus* drift controversy? The determinate changes in allelic frequencies in the cage populations, leading to final frequencies very close to the Groningen base population, strongly point to some kind of balancing selection. Neutrality of the alleles would only lead to small chance fluctuations around the initial values, as population sizes amount to several thousands. When the observed numbers of genotypes, from which the allelic frequencies in Table 1 are calculated, are compared with the numbers, expected on the basis of the Hardy–Weinberg equilibrium, significant deviations ($P<0.05$) are found in seven of eighteen cases. In all these cases, the actual number of heterozygotes exceeds the expected number. Detailed fitness measurements, now in progress, will elucidate the exact nature of the selection process. It cannot be stated with absolute certainty, however, from these experiments that the *Adh*-locus itself is involved, instead of a closely linked locus. In that case, an initial linkage disequilibrium between the *Adh*-locus and the linked locus may bring about the described reaction. The rapid rise in F-frequency, however, found in the vial populations with medium supplemented with ethanol, which afterwards proved to be a general phenomenon for polymorphic *D. melanogaster* populations from different geographic origins (Van Delden, unpublished), strongly suggests a reaction of the *Adh*-locus itself on a relevant environmental change. Recently[13], comparable systematic changes in allelic frequencies, follow-

Table 2 Cumulative extinction percentages in different environmental conditions after eight generations

Population type	Environments								
	1	2	3*	4	5	6	7†	8	9
Monomorphic F	4	90	100	36	28	94	8	8	28
Monomorphic S	0	94	100	24	62	92	32	14	2
Polymorphic (0.20 F)	10	82	100	36	30	54	12	30	10
Polymorphic (0.80 F)	6	78	100	42	20	66	12	20	18
χ^2‡	5.5	6.7		3.8	22.6§	32.4§	13.1§	8.9¶	15.0§

* Complete extinction after four generations.
† Extinction percentages after three generations.
‡ χ^2 for independence.
§ $P<0.01$.
¶ $P<0.05$

The following environmental conditions were applied. 1, Control, temperature 25° C, 70% R.H., food medium consisting of 1,000 ml water, 19 g agar, 54 g sucrose, 32 g yeast, 13 ml nipagin solution (10 g nipagin in 100 ml ethanol 96%); 2, yeast amount reduced to 5 g; 3, yeast amount reduced to 5 g, medium supplemented with 10% ethanol; 4, sucrose content reduced to 25 g, after 6 generations further reduced to 12.5 g; 5, medium supplemented with 20% ethanol; 6, temperature 29.8° C; 7, temperature 15° C; 8, 35% R.H.; 9, 95–100% R.H.

For conditions 2–9 only differences relative to the control are listed.

ing changes in the composition of the food, were found for the amylase locus in *D. melanogaster*.

The extinction experiments show that the genetic composition of a population concerning the *Adh*-locus, at least in some environments, strongly influences the chance of population survival. Different environments favour different population types. Experimental data on the relative success of populations, differing in genetic composition, are scarce. Studies on third chromosome inversion polymorphism in *D. pseudoobscura* have shown that polymorphic populations are superior to monomorphic ones, in such characteristics as population size and innate capacity of increase[14,15]. Though in the present experiment the polymorphic population types show the lowest extinction in the high temperature environment, this is, as the data show, no general phenomenon holding for all environments. It is interesting to note that for some environmental conditions there is a relation between the extinction rate of population types and the specific activities of the *Adh*-genotypes concerned. Gibson[16] found that after heat treatment of larval extracts, the heterozygotes possess the highest remaining activity, as in our experiments, polymorphic populations show the lowest extinction rates. Further, he found that when larvae are cultured on food, supplemented with ethanol, the SS genotypes, which have the lowest activity in normal conditions, show less increase in activity than the other genotypes. This corresponds with our findings that the SS monomorphic populations show the highest extinction rates on this medium.

Table 3 Change in frequencies of the *Adh*^{F}-allele in polymorphic vial populations, kept in different environments

Environments*	Generation	0.20 F populations	0.80 F populations
1	7	0.42 (250)	0.65 (234)
2	4	0.43 (168)	0.70 (192)
3	3	0.42 (84)	0.62 (62)
5	5	0.65 (224)	0.87 (243)
6	5	0.42 (282)	0.67 (300)

* As in Table 2.
Sample size given in parentheses.

Although the results of our experiments strongly point to the occurrence of some kind of balancing selection, we further envisage a situation in which selection between populations is working, superimposed on the selection process within populations. Lewontin[17] has discussed the relations between such intra- and interpopulation selection, and stressed the importance of interpopulation selection for the evolution of genetic systems The fossil record shows that extinction of species and higher systematic categories is a widespread phenomenon and inextricably linked with the evolutionary process, but we do not know if extinction of local populations, and more specifically of *Drosophila* populations, plays an important role in nature. It is quite probable, however, that extreme temperature and humidity conditions, shortage of certain food components, and excess of harmful products, for example, create a severe stress on local populations, leading to complete extinction of part of them. If the probability of extinction is correlated with genotype composition, the random succession of a number of differing extreme environments would provide polymorphic populations, segregating for all genotypes, with the best chances of long-term survival. A survey of natural populations would then show most of them to be polymorphic. Relevant to this conclusion is the finding that for experimental *D. willistoni* populations genetic variability is higher in populations kept in heterogeneous environments than in populations in more constant environments[18]. It is tempting, though speculative, to assume that when polymorphism is the overall best adaptive strategy in varying environments, the development of systems providing balanced polymorphism will be stimulated.

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Synergistic Response between Thymus and Lymph Node Cells in the Mixed Lymphocyte Culture

In rodents the mixed lymphocyte culture (MLC) reaction probably represents an *in vitro* model of the graft *versus* host (GvH) response[1]. Thymus-dependent lymphoid cells (T cells) are thought to be responsible for both reactions[2,3]. Cantor and Asofsky[4,5] presented evidence for the synergistic interaction of two thymus-dependent lymphocyte populations in the GvH response. In their studies one cell type (amplifier cells or T_2 cells) was obtained from the peripheral circulation, the other type (precursor cell or T_1 cell) was found in greatest concentration in the thymus and spleen. A mixture of thymocytes and peripheral blood lymphocytes produced a GvH reaction significantly greater than was expected from summation of the separate reactivities of the components of the mixture. This 'synergy' occurred only when both cell populations had been obtained from donors allogeneic to the host[4].

We present here evidence that the *in vitro* 'one way' MLC reaction can also be used to show 'synergy' between subpopulations of lymphocytes. For our experiments, 4 to 8-month-old C57Bl/6J (B6) and CBA/6J (CBA) and 2-month-old (B6×CBA) F_1-hybrid males were used. Thymuses, lymph nodes (LN), and spleens were removed aseptically. Cell suspensions free of debris were prepared in HEPES-buffered medium RPMI/1640 (Gibco) containing 1% antibiotic-antimycotic-mixture (Gibco) and 7% pooled, heat-inactivated normal human serum. Spleen cell suspensions were treated with Tris-buffered ammonium chloride to remove contaminating red blood cells[6]. For the MLC reaction, 0.1 ml of the responding cell suspension was incubated for 48 h at 37° C in a humidified atmosphere with 0.1 ml of mitomycin C-treated (mito-treated)[7] stimulating

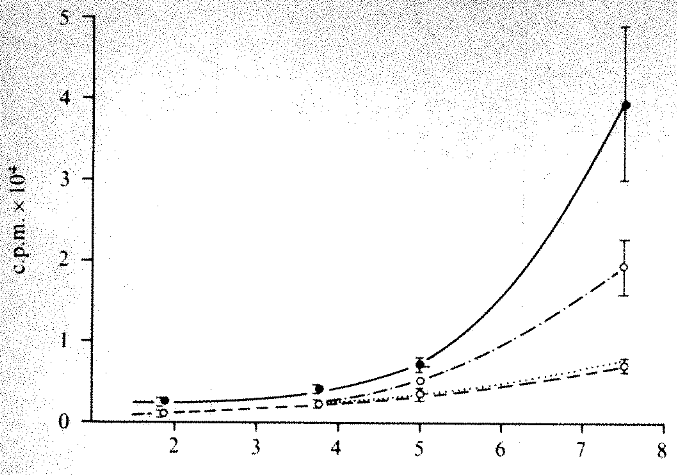

Fig. 1 ^{3}H-thymidine uptake (in c.p.m.) of LN cells (——), thymocytes (- - -), and a mixture of 20% LN cells 80% thymocytes (— · — · —), from 6-month-old B6 mice, in relation to the concentration of responding cells. The ratio of stimulating cells (mito-treated CBA spleen cells) to responding cells was 1 to 1 in each case. The 'expected' reactivity for the LN-thymocyte mixture (. . .) was calculated from the reactivity curves for equivalent absolute numbers of LN cells and thymocytes. This calculated sum of c.p.m. values of the participating partner cells was significantly less than the observed ^{3}H-thymidine uptake of the mixture. The vertical bars in this and the other figures represent standard error.

cell suspension in the U-shaped cups of titration plates (Scientific Products; 220–24A Disco U titration plates) which had been sealed with covers (Linbro, 35 PSM). Then 3 μCi of ^{3}H-thymidine (Schwarz Mann; specific activity 1.0 Ci mmol^{-1}) in 0.1 ml of serum-free medium were added to each cup. After a further 15 to 18 h of incubation, the reaction was stopped by chilling, the reaction mixture transferred to centrifuge tubes, washed twice in 5% trichloroacetic acid, once in 100% methanol, transferred quantitatively to counting vials, and evaporated to dryness. Scintillation liquid (5 g PPO and 100 mg POPOP l^{-1}) containing 3% solubiliser (Nuclear-Chicago Corp.) was added to each tube and the activity measured in a Beckman model LS-250 liquid scintillation counter. The amounts of ^{3}H-thymidine uptake in tubes containing only synergistic partner cell were used as controls and subtracted from the experimental (allogeneic) values. Final values were expressed as c.p.m. × 10^4. Each value in Figs 1, 2 and 3 and Table 1 represents the arithmetic mean with standard error from four separate cultures. Student's t test was used for statistical comparisons. When 'expected' compared with mean values were compared the s.e. of the observed value was used for both means: this introduced a slight conservative bias in the probability values.

Table 1 MLC reactivity of LN and thymus cells and of parental and parental–hybrid mixture

Source of resp. cells.	Strain	Cell concentration (× 10^6)	c.p.m. Allogeneic	c.p.m. Syngeneic
LN	B6	7.5	114,949 ± 5,400	1,232
LN	B6	3.75	67,740 ± 8,200	484
LN	B6	1.875	16,467 ± 6,450	—
LN	B6	0.9375	4,411 ± 1,900	—
Thymus	B6	7.5	11,619 ± 620	289
Thymus	B6	3.75	1,425 ± 200	248
LN 20% Thymus 80%	B6 B6	7.5	19,000*	—
LN 20% Thymus 80%	B6 B6	7.5	42,274 ± 4,800	1,308
LN 20% Thymus 80%	B6 × CBA B6 × CBA	7.5	4,494 ± 700	727
LN 20% Thymus 80%	B6 B6 × CBA	7.5	31,569 ± 2,800	2,223
LN 20% Thymus 80%	B6 × CBA B6	7.5	13,256 ± 4,600	2,179

* B6 LN/B6 thymus, expected compared with observed, $P<0.01$; B6 LN/B6 thymus expected compared with B6 LN/(B6 × CBA) thymus observed, $P<0.02$; B6 LN/B6 thymus observed compared with B6 LN/(B6 × CBA) thymus observed, $P=0.01$.

† Expected value for the LN-thymus mixture.

MLC reactivities of B6-LN cells and B6 thymocytes at different cell concentrations, and of 20/80 LN-thymocyte mixtures at a total concentration of 7.5 × 10^6 cells per ml. The cells in the mixtures were derived from B6 mice or (B6 × CBA) hybrids. Mito-treated CBA spleen cells (7.5 × 10^6 cells per ml) served as stimulating cells. An optimal (synergistic) response was observed only when both partner cells could be stimulated, that is they were from B6 mice.

Figure 1 shows results for different concentrations of responding cells of separate MLC-reactions for LN cells, thymocytes, and a mixture containing 20% LN cells and 80% thymocytes from B6 mice. Mito-treated CBA spleen cells were used as stimulating cells. LN cells showed much greater ^{3}H-thymidine uptake than thymocytes. The LN-thymocyte mixtures reacted more strongly than expected: according to the curves, 1.5 × 10^6 LN cells ml^{-1} (=20% of 7.5 × 10^6) should give about 0.25 × 10^4 c.p.m. and 6 × 10^6 thymocytes ml^{-1} (=80% of 7.5 × 10^6) about 0.4 × 10^4 c.p.m. The sum of both c.p.m. values would therefore equal 0.65 c.p.m. Actually, 7.5 × 10^6 cells of the 20%/80% mixture showed 1.95 × 10^4 c.p.m., which is about three times the 'expected' value ($P=0.01$).

In the experiment shown in Fig. 1 equal total numbers of stimulating and responding cells were present in each cup. It might therefore be argued that the LN cell component of the LN-thymocyte mixture received extra stimulation from the increased numbers of stimulating CBA cells per LN cells present in the mixture. The apparent 'synergy' might in this case be specious. Figure 2 shows results of MLC-reactions in which the concentration of stimulating cells in each cup was kept constant, and so the ratio of responding cells to stimulating cells increased with increasing concentration of responding cells. Here the reactivity of the LN-thymocyte mixture at the responding cell concentration of 7.5 × 10^6 ml^{-1} was about four times the 'expected' value ($P=0.05$).

Figure 3 includes results of an experiment in which the ratio of LN to thymus cells in the responding cell mixture was allowed to vary but the total concentration of the responding cells was kept at 7.5 × 10^6 cells ml^{-1} for each cup. It also shows the results when one of the cells in the LN-thymocyte mixture was inhibited by previous treatment with mitomycin. Separate experiments demonstrated that mito-treated syngeneic partner cells behaved like 'innocent bystanders' and did not affect the reactivity of the untreated partner cells. The 'expected' curve in Fig. 3 was constructed from the reactivity curves of the mixtures LN$_{\text{mito}}$-thymocyte and LN-thymocyte$_{\text{mito}}$. The untreated LN-thymocyte mixture demonstrated a statistically significant synergistic effect when LN cells constituted 20 to 40% of the mixture. Mitomycin-inhibited cells were not able to synergise.

Table 1 gives results of experiments comparing MLC reactions of LN and thymus cells of B6 mice as responding cells with reactions where one or both responding cell came from (B6 × CBA) F$_1$ hybrids. Stimulating cells were CBA in all cases. Now F$_1$ hybrid cells are not normally stimulated in the MLC reaction. These experiments were therefore set up to determine if F$_1$ hybrid cells could participate in synergy. The highest ^{3}H-thymidine uptake (42,274 c.p.m.; control not subtracted) for any of the mixtures was seen when both lymph node cells and thymocytes were derived from B6 mice (Table 1). The LN-thymocyte mixture in which both cells was derived from the hybrids showed the lowest c.p.m. value (4,494 c.p.m.). This value was, however, higher than for a syngeneic control in which the same LN-thymocyte mixture was allowed to react against mito-treated

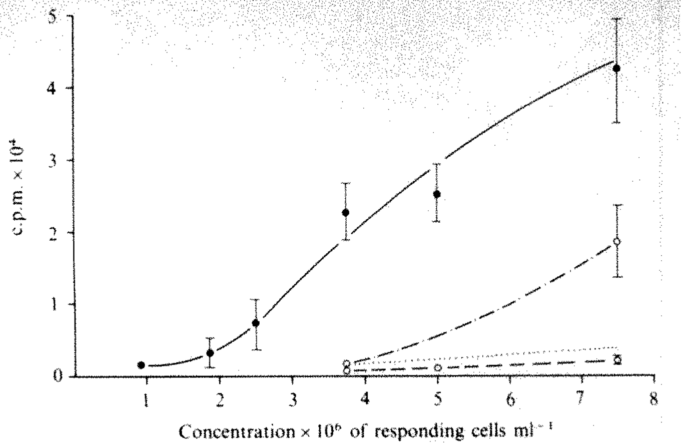

Fig. 2 Legend as for Fig. 1, except that the concentration of the stimulating mito-treated CBA spleen cells was kept constant at 7.5×10^6 cells ml^{-1}; and so the ratio of responding to stimulating cells increased with increasing concentration of responding cells. The reactivity of the LN-thymocyte mixture at the responding cell concentration of 7.5×10^6 cells ml^{-1} was about four times the 'expected' value.

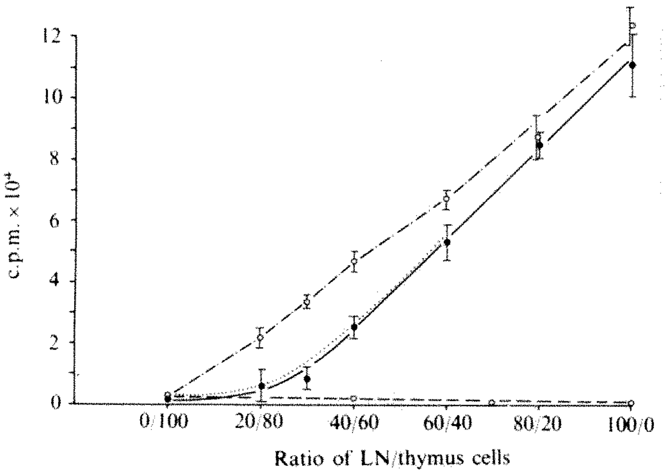

Fig. 3 MLC reactivities of LN cells and thymocytes (— · — · —), LN cells and $thymocytes_{mito}$ (——) and thymocytes and LN $cells_{mito}$ (- - -) at varying mixture ratios but with a constant total concentration of responding cells (8×10^6 cells ml^{-1}). The expected reactivities for the LN-thymus mixture (. . .) were calculated from the reactivity curves for $LN\text{-}thymocytes_{mito}$ and $thymocytes\text{-}LN_{mito}$. The observed reactivities of the LN-thymocyte mixtures at ratios of 20:80, 30:70 and 40:60, respectively, were significantly greater than these 'expected' reactivities. Synergy was not observed when one participating partner of a mixture had been treated with mitomycin. The concentration of stimulating mito-treated CBA spleen cells was 8×10^6 ml^{-1}.

syngeneic (B6×CBA) F_1 hybrid cells (c.p.m.=4.494 for allogeneic compared with 727 for syngeneic cells, Table 1). This suggests that a weak reaction occurred in the combination (B6×CBA) LN-thymocyte responding cells compared with mito-treated CBA spleen cells. Boehmer and Adams[8] found that mito-treated CBA spleen cells stimulated syngeneic CBA thymus cells weakly. It seems likely that the thymocyte component of the (B6×CBA) LN-thymocyte mixture might have been stimulated by CBA spleen cells. (Boehmer and Adams[8] did not find a syngeneic interaction of cells derived from B6 mice, but only from CBA mice.) The (B6×CBA) LN and B6-thymocyte mixture showed reactivity numerically higher but not statistically significantly different from pure B6 thymocytes (Table 1). The B6-LN-(B6×CBA)-thymocyte mixture exceeded the 'expected' reactivity of the B6-LN-B6-thymocyte mixture. This might be explained by a reaction of hybrid thymocytes with mito-treated CBA spleen cells, as noted above. This reaction, however, was still significantly lower ($P<0.02$) than the actually observed (synergistic) ^{3}H-thymidine uptake of the B6-LN-thymocyte mixture. It is additionally possible that in the parental-hybrid mixtures the parental cells received some stimulation by the extra antigens of the hybrid component[9], which also could account for somewhat higher syngeneic control values (2,223 and 2,179 c.p.m., Table 1). These values were somewhat higher than the control values for pure LN cells or pure thymus suspensions. Thus, in the allogeneic system parental cells of a parental-hybrid mixture might be stimulated to a mild degree both by the mito-treated CBA spleen cells and by the cells of the hybrid partner. In general, however, our experiment indicates that an optimal (synergistic) response occurs only when both partners of the responding LN-thymocyte mixtures recognise the stimulating cells as foreign.

Our finding of a synergistic response involving LN and thymus cells in the MLC reaction seems analogous to the results of Cantor and Asofsky[3-5] with the GvH reaction. A maximum response occurred in the LN-thymocyte mixture when the LN cells comprised 20 to 40% of the responding cell population. Mitomycin-inhibited partner cells were not able to participate in synergy. Synergy did not seem to occur to any great degree unless both cell populations were obtained from donors allogeneic to the stimulating cells. In experiments not detailed here treatment of LN cells with anti-θ serum abolished synergy. The MLC reaction is considered by most investigators to be the *in vitro* counterpart of the GvH reaction. Our results may be interpreted to support this view and suggest that for optimal responses the same cell subpopulations may interact both in the MLC reaction and the GvH reaction.

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Proliferative Capacity of Human Alveolar Macrophage

THE alveolar macrophage plays a crucial role in the pulmonary ecology and is a first line of cellular defence for the lung[1,2]. Although animal studies indicate that pulmonary macrophages originate from the bone marrow[3-5], little is known of the origin or fate of these cells in man. Cigarette smokers, however, are known to have substantially increased numbers of macrophages in the lung[1,6] and the pulmonary macrophages in smokers demonstrate characteristic morphologic and functional attributes[1,2,7,8].

To determine whether human alveolar macrophages have proliferative capacity, we obtained cells by bronchopulmonary lavage from normal cigarette smokers and a non-smoker and performed *in vitro* tritiated thymidine labelling and culture studies. The data obtained indicate that some of these cells have proliferative capacity and that the lung macrophage population may therefore be locally self-sustaining.

Pulmonary cells were obtained from five normal volunteers (four cigarette smokers and one non-smoker) by the procedure of bronchopulmonary lavage as previously described[9]. Briefly, under local anaesthesia and fluoroscopic control, a catheter was placed into a lower lobe bronchus and lavage performed with 300 ml of normal saline. The cells were collected in Hank's balanced salt solution containing 20% foetal calf serum and 200 U of penicillin and 100 μg streptomycin ml^{-1}. The cells were recovered by centrifugation at 150g for 7 min, washed twice, and suspended in McCoy's 5A medium with 20% foetal calf serum and antibiotics[10].

Labelling studies were performed 1 to 3 h after pulmonary lavage by exposing the cells to ^{3}H-thymidine (specific activity 2 Ci mmol^{-1}) at a concentration of 1 mCi ml^{-1} of cell suspension for 1 h (ref. 11). In blocking experiments, cytosine arabinoside (final concentration, 10^{-5} M) or hydroxyurea (10^{-3} M) was added to the cell suspension 30 min before radiothymidine exposure[12]. After incubation with ^{3}H-thymidine, the cells were washed twice in complete tissue culture medium and deposited on glass slides with a cytocentrifuge. Slides coated with Kodak NTB emulsion were exposed for 7 d, developed, and stained with Giemsa[11]. Labelling indices were expressed as % macrophages labelled with a minimum count of 500 cells.

Pulmonary cells were cultured using the *in vitro* diffusion chamber technique previously described[10,11]. Differential cell counts were performed on Giemsa stained specimens and macrophage identification was also based on electron microscopy, histochemical reaction for lipase (alpha naphthyl butyrate substrate)[13] and tests of functional capacity including phagocytosis of yeast and bacteria and tests for the presence of cell surface receptors for immunoglobulin[14]. Lymphocytes were identified by characteristic morphology at the light and electron microscopic level and transformed lymphocytes by intense staining with methyl green pyronin.

Table 1 summarises the observed ^{3}H-thymidine labelling indices. The percentage of macrophages incorporating label was found to vary between 0.35 and 1.25. The labelled cells generally had the morphology of immature macrophages[2] but occasional mature macrophages also incorporated thymidine (Fig. 1). Some of the labelled cells were seen to contain smoker particles thereby substantiating that label was taken up by phagocytic cells which had been residing in the airways (Fig. 1). Tritiated thymidine uptake was blocked more than 90% by either cytosine arabinoside or hydroxyurea indicating that labelling resulted primarily from scheduled DNA synthesis rather than DNA repair[12]. Occasional mitotic figures (Fig. 2) in macrophages were also observed.

Table 1 Pulmonary macrophage recovery and ^{3}H-thymidine labelling

Subject		Viable cells recovered ($\times 10^6$)	Macrophages (%)	Total macrophages ($\times 10^6$)	Macrophage labelling index (%)
Smokers	1	45	99	45	0.75
	2	62	97	60	1.25
	3	84	98	82	0.35
	4	160	92	147	0.5
Non-smoker	5	11	72	7.9	0.35

Differential and total cell counts (Table 1) confirmed that pulmonary macrophages retrievable by bronchopulmonary lavage are markedly increased in cigarette smokers. These cells had the typical morphology of smoker macrophages as

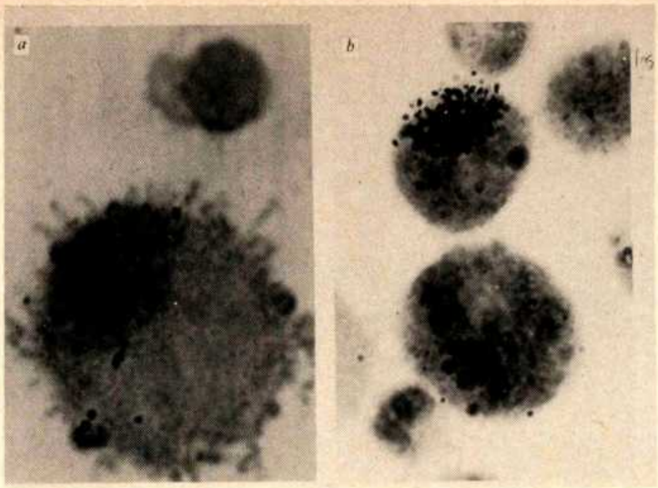

Fig. 1 *a*, Mature macrophage which has incorporated ^{3}H-thymidine and a small lymphocyte from non-smoker; *b*, two macrophages with smoker inclusions. Upper cell has heavy nuclear label.

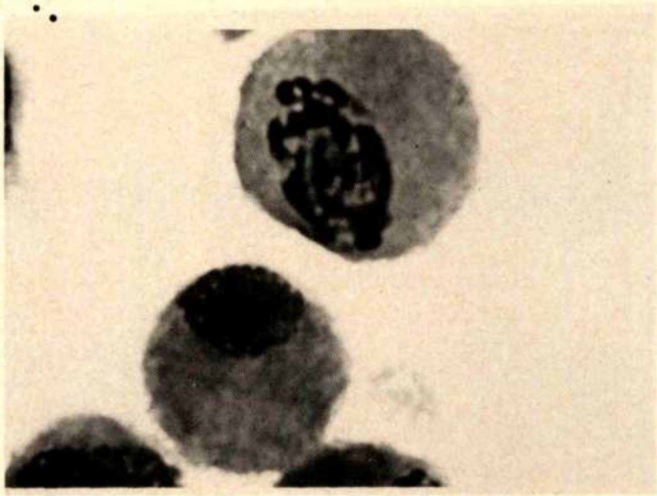

Fig. 2 Mitotic figure in pulmonary alveolar macrophage and non-mitotic cell of approximately the same maturity.

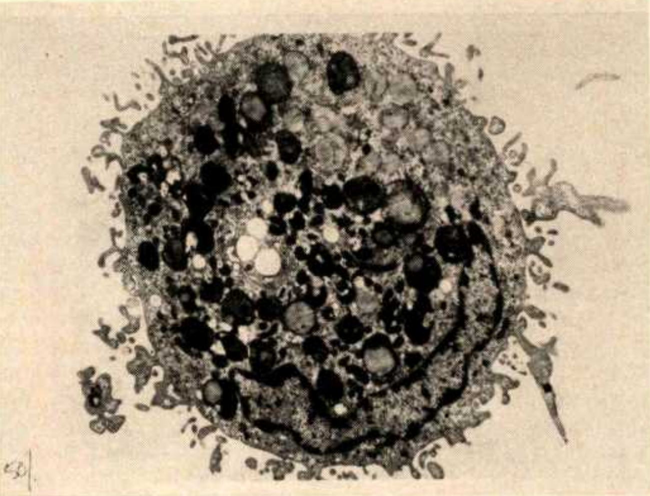

Fig. 3 Electron micrograph of pulmonary macrophage in culture at 14 d. Lipid inclusions and smoker particles are clearly seen.

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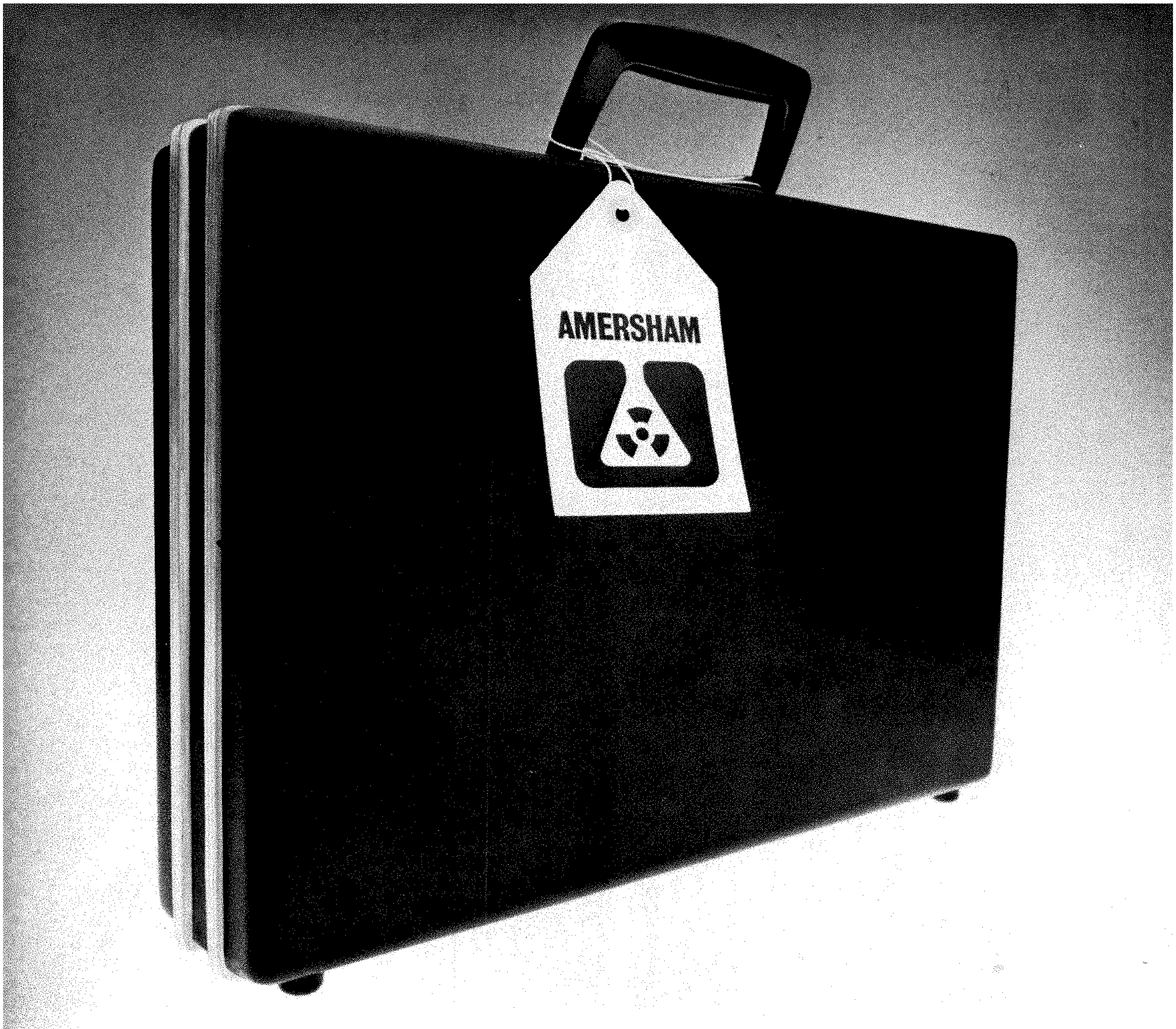

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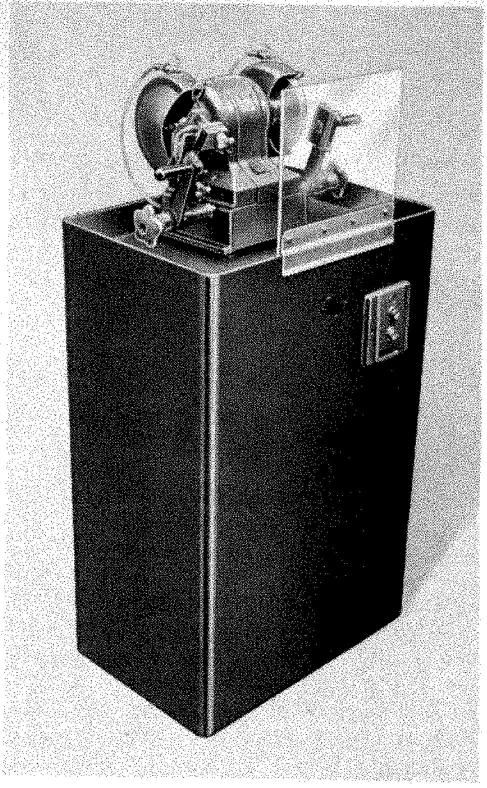

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viewed by light and electron microscopy[7,8] and their morphological characteristics were maintained *in vitro* for several weeks (Fig. 3). They retained phagocytic capacity in culture for periods in excess of 50 d. Low labelling was also observed in macrophages maintained *in vitro* for up to 10 d and then exposed to ^{3}H-thymidine for 1 h, indicating that these cells continued to synthesise DNA while in culture. In three experiments, the cells from smokers were exposed for 1 h to ^{3}H-thymidine at the initiation of culture and then washed and grown in medium containing 10^{-5} M thymidine[11]. In these cultures, labelled mature macrophages were observed for 5 to 14 d *in vitro*. Replicating transformed lymphocytes were also seen for several weeks in cultures from the non-smoker, indicating that lymphocytes resident in the lung are also capable of proliferation.

There is evidence from animal studies that pulmonary macrophages originate in the bone marrow[3-5]. Data are also available indicating that both the pulmonary alveolar macrophage and the hepatic macrophage can replicate *in situ*[15,16]. It is presumed that in man the alveolar macrophages originate in the bone marrow and that the lung macrophage population is supported by an influx of monocytes from the blood. This concept raises important questions regarding the fate of the alveolar macrophage population in patients with bone marrow failure and severe leukopaenia. It also implies that needed increases in lung macrophages are dependent on a recruitment mechanism from the bone marrow[10].

Our studies were undertaken to determine if the human pulmonary macrophage has proliferative capacity. The data obtained indicate that a small percentage of macrophages resident in the lung incorporate ^{3}H-thymidine. This incorporation probably represents pre-replicative DNA synthesis for the following reasons: labelling was heavy and limited to a small percentage of cells; uptake of ^{3}H-thymidine was blocked by hydroxyurea and cytosine arabinoside and mitotic figures were observed.

The long life of the pulmonary macrophage in culture lends support to the concept that these cells may be quite long-lived *in vivo*[2,18]. A small growth fraction, therefore, as suggested by the low labelling index, could be sufficient to maintain a stable cell population without the need for an influx of mononuclear cells from the blood. This is consistent with data obtained for the pulmonary and hepatic macrophage in animals[16,17] and general information regarding the proliferative capacity of mononuclear phagocytes[18]. Local pulmonary macrophage replication has also been shown to occur in rats exposed to NO_2 (ref. 15).

Our studies indicate that the human pulmonary macrophage is capable of replication. The macrophage compartment could, therefore, be sustained by local cell proliferation as well as by an influx of cells from the peripheral blood and by recruitment from the bone marrow[10].

We thank Dr Martin J. Cline for his help.

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Maintained Function of Foreign Synapses on Hyperinnervated Skeletal Muscle Fibres of the Rat

MAMMALIAN skeletal muscle fibres normally receive input from single nerve fibres only, but will accept additional innervation from a foreign nerve if the original nerve is cut[1,2] or anaesthetised[3]. We have been interested in the question of whether the extra innervation is repressed or displaced as the original innervation is re-established. Such synaptic repression has been suggested by Mark and collaborators[4,5] for lower vertebrates and has recently attracted great interest[6,7].

The superficial fibular nerve, which normally innervates flexor muscles in the lower leg, was used as the foreign nerve in our experiments. It was dissected, cut peripherally, and transplanted into the proximal surface of the soleus muscle of young rats. Two weeks later the soleus was deprived of its normal nerve supply by cutting or crushing the tibial nerve in the popliteal fossa, about 15 mm from the muscle. The foreign nerve then formed synaptic contacts on soleus muscle fibres during the several weeks required for the original nerve to regenerate. Between 2 and 5 weeks after the denervation of the soleus, before regeneration of the tibial nerve was complete, we determined the extent of foreign innervation by measuring the tension developed in the soleus muscle after giving single shocks to the fibular nerve. This was done in the intact, anaesthetised animal with minimal dissection and muscle exposure. The knee joint was fixed by steel pins. A thread connected the triceps tendon to an isometric transducer and the fibular nerve was exposed for stimulation in the thigh. The deep fibular nerve branches were cut at this time to avoid interference from contraction of flexor muscles. After determining soleus twitch tension to foreign nerve stimulation and confirming early signs of re-innervation by the original nerve, wounds were stitched and the animals recovered without complication.

Terminal experiments were performed from 3 to 6 months later. First, soleus twitch tension to fibular nerve stimulation was determined in the intact leg, as above. Then the soleus muscle with transplanted and original nerves was dissected free and examined in a chamber perfused with oxygenated rat Ringer's solution. The measurement *in vitro* confirmed the extent of foreign nerve innervation of the muscle and showed the degree of re-innervation by the original nerve.

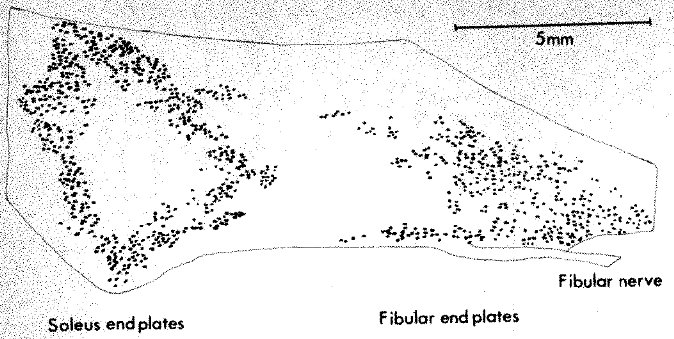

Fig. 1 Distribution of original and foreign endplates in a doubly innervated soleus muscle. The drawing outlines the proximal two-thirds of the muscle and gives the position of the end-plates as seen in a projection of a whole mount, esterase-stained preparation. The upper one-third of the muscle did not contain any foreign endplates.

In all eight rats the twitch tension *in vivo* in the soleus muscle produced by the transplanted foreign nerve during the final experiment was greater than, or in one case equal to, the one measured earlier. The average peak value was 6.5 g (range 1.4 to 12.5 g), which was approximately one-third of the tension produced by direct stimulation of the muscles. This was an average increase of 67% over the value obtained at the interim experiment (3.9 g, range 1.4 to 7 g). There was thus no suggestion of a repression of the foreign synapses, in fact, the fibular innervation increased moderately in spite of the increasing re-innervation by the original nerve supply, and the two groups of synapses remained functional for at least several months. In other experiments where the interim experiment was not performed, we have seen extensive functional foreign innervation even 9 months after re-innervation by the original nerve.

Intracellular recordings were made to study the innervation of individual muscle fibres on the dorsal surface of the muscle. In all muscles a substantial fraction of all fibres examined (12 to 38% after cutting the original nerve, 32 to 57% after crushing it) were innervated by both the foreign and the original nerves, that is, they received dual innervation. Functional foreign synapses can therefore co-exist with original innervation on single muscle fibres for prolonged periods of time. Further, extensive re-innervation by the original nerve was not prevented in these experiments.

The spatial extent of foreign innervation could be judged by sampling with a microelectrode and by visual inspection during nerve stimulation. In most muscles, fibres innervated by the foreign nerve were located only in the lateral one-third to two-thirds of the muscle (Fig. 1). Within this zone, 92±8% (mean±one s.d.) of the superficial fibres had foreign innervation. Further, the functional border, usually quite sharp, coincided closely with the anatomically defined border demonstrated by staining for ACh-esterase[8] and by the abrupt termination of visible fibular nerve branches on the surface of the muscle. Accordingly, the presence of a substantial number of repressed foreign synapses in these muscles is unlikely.

The denervation of the original nerve supply to the muscle was usually performed in the thigh and included denervation of all the other muscles innervated by the tibial nerve. The regenerated 'original' nerve probably contained nerve fibres not originally belonging to the soleus muscle. True soleus nerve fibres might be more efficient in suppressing foreign innervation. So, in three of the eight rats we denervated the muscle by cutting only the soleus nerve between the gastrocnemius and the soleus muscle. In all these animals the soleus muscle was innervated by the foreign nerve in both the interim and the final experiments, and the situation in the soleus muscle was indistinguishable from that in the other rats.

Our findings therefore suggest that foreign synapses in adult mammalian muscle are not repressed or displaced. These results are in sharp contrast to those reported for similar experiments in fish and salamanders. Inappropriate neuromuscular synapses in these lower vertebrates are apparently repressed after muscles are re-innervated by their original nerves[4,5]. In adult mammalian muscle, however, foreign synapses can remain functional for extended periods of time even after correct innervation has been restored.

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Behavioural Effects of *in utero* Administration of Morphine

ALTHOUGH numerous studies have been concerned with the behavioural effects of opiates, relatively little attention has been paid to the offspring of females receiving an opiate during pregnancy. The matured offspring's response to stress and to the same drug are important issues in terms of current drug abuse and potential widespread application of opiate maintenance programmes. Since morphine is readily transferred across both placental and lacteal barriers[1,2], we have studied the analgesic response to morphine in the offspring of mice receiving the drug during gestation and lactation.

Adult female CF 1 mice, housed in individual cages, were mated with adult CF 1 males for 1 week. After removal of the males, half the females were given a sweetened (saccharin, $0.4\ \mathrm{g\ l^{-1}}$) morphine sulphate solution ($0.5\ \mathrm{g\ l^{-1}}$) instead of regular drinking water. Fluid intake (measured in graduated drinking tubes) in the drug group was no different from water intake in the control group. Approximately 2 weeks later, seven of the females had given birth to a total of forty-one mice, twenty in the drug group. Sex ratios were similar for the two groups. The morphine drinkers were retained on the oral drug solution for the first 2 weeks of nursing to avoid withdrawal which might upset lactation and introduce a nutritional variable. When the litters were 2 weeks old, the morphine was withdrawn and the offspring weaned, assuring that they would have had no exposure to morphine except through the maternal placenta or mammary glands. When they were 2.5 months old, the mice born to both the control and drug groups were tested for shock-elicited escape behaviour on day 1 and for morphine sensitivity on day 2.

The escape apparatus was a Plexiglas trough 10 inches long, 6 inches high, 1.5 inches wide on the floor and 4 inches wide at the top. The bottom of the trough consisted of two metal plates, 2–3 mm apart, arranged so that the animal continually bridged the two plates and received a $160\ \mu\mathrm{A}$ foot shock for as long as it remained in the apparatus[3]. On day 1, all mice were placed in the trough individually and the latency to jump 6 inches up onto a Plexiglass platform was recorded. If the subject did not jump up within 30 s, it was removed and a score of 30+ was recorded. The procedure was identical on day 2, except that all mice received 25 mg kg^{-1} morphine sulphate intraperitoneally 30 min before testing.

Table 1 Response to foot shock and sensitivity to morphine

	Median escape latency (s)	
Group	Day 1	Day 2
Drug *in utero*	1.15*	8.80†
Control	3.80	30+

*, † Significantly less than control at $P<0.01$ and 0.02, respectively (Wilcoxon rank sum test, two tailed).

On day 1 (see Table 1) mice which had been exposed to morphine *in utero* escaped the shock with significantly lower latencies than the control offspring. On day 2, both groups had significantly ($P<0.001$) higher latencies than on day 1, indicating an analgesic effect of the drug; but the effect was significantly less in the group whose mothers had been drinking morphine. This implies tolerance to the analgesic effect of the drug in the latter group.

The results of the day 1 experiment suggest that litters exposed to morphine *in utero* may exhibit an altered response to stress; this alteration may occur as a consequence of an increase in pain sensitivity or a hyper-reactivity to the same degree of pain. This finding may be relevant to a hypothetical role of stress in drug addiction[4]. The day 2 test for morphine sensitivity revealed tolerance to the drug. Tolerance to morphine has been observed a year after administration of a single dose to rats[5]. This study indicates that tolerance can occur in mature mice if the drug is administered through the mother during gestation and lactation. 'Personal' morphine experience with long-term consequences can therefore be similarly acquired by placental, lacteal and other routes of administration[6]. Since passive administration of morphine in adult animals can increase the probability of subsequent addiction[7], the fact that the placental route of administration of morphine is similar to other routes seems a critical issue in opiate addiction during pregnancy.

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In vitro Transfer of Cell-mediated Immunity to Hepatitis B Antigen with RNA

THE immunological basis of the chronic carrier state for hepatitis B antigen (HBAg) in man remains ill-defined. Knowledge of experimental immunity to HBAg in animal models may provide an insight into the concepts and means of terminating the human chronic carrier state, which seems to be a reservoir for transmission of type B viral hepatitis. The *in vitro* and *in vivo* measurements of cell-mediated immunity to HBAg in guinea pigs have been previously reported[1,3]. The migration inhibition of peritoneal macrophages in the presence of HBAg is an excellent correlate of delayed hypersensitivity to HBAg in guinea pigs[2]. We now report the *in vitro* transfer of cell-mediated immunity to non-immune cells as measured by inhibition of migration in the presence of HBAg, using RNA extracted from the lymphoid tissue of guinea pigs immunised with HBAg.

Adult albino guinea pigs of Hartley strain weighing 600–700 g were immunised by injecting 100 µg of purified HBAg subtype *ad* mixed with complete Freund's adjuvant (CFA) (ref. 2). Similarly, control animals were sensitised with CFA alone. Three weeks following immunisation, the animals were skin tested with HBAg. A strong delayed hypersensitivity reaction occurred in immunised animals, whereas no reaction to HBAg was observed in control animals. The spleen and enlarged lymph nodes of each animal were excised, and subjected to phenol extraction of RNA according to the method previously described[4]. The RNA concentration was spectrophotometrically measured and only the extracts with optical density ratios between 1.9 and 2.1 when measured at 260 nm and 280 nm wavelengths[5,6] were employed in these studies.

The RNA extracts from animals immunised with HBAg (referred to as IRNA) and RNA from those immunised without HBAg (referred to as NRNA) were extensively dialysed against 0.3 M acetate buffer (*p*H 5.0) at 4° C, reprecipitated with prechilled 95% ethanol and dissolved in medium 199. The solutions of IRNA and NRNA at a concentration of 1 mg ml^{-1} ($E^{0.1\%}_{1\,\mathrm{cm}}=22.0$) were used for experiments of transfer of immunity. The RNA was devoid of HBAg or anti-HBAg activity when tested by radioimmunoassay (Austria™, Abbott Laboratories, Chicago) and haemagglutination assay respectively[7,8].

Table 1 Transfer of Cell-mediated Immunity to HBAg Measured by Migration Inhibition of Peritoneal Exudate Cells

Treatment of normal guinea pig peritoneal exudate cells with	Migration indices
IRNA	0.10; 0.33; 0.33
NRNA	0.98; 1.00; 0.95
IRNA treated with RNase*	0.94; 0.98
NRNA treated with RNase	0.98; 0.96

The capacity of IRNA to transfer experimental *in vitro* immunity was destroyed by its treatment with RNase.

In separate reaction mixtures 0.5 ml of medium 199 containing 7×10^7 peritoneal exudate cells from a normal guinea pig was mixed with 0.5 ml solution of each IRNA and NRNA, and incubated for 30 min at 37° C. The treated cells were washed three times with Hanks balanced salt solution and packed in capillary tubes for migration inhibition assays carried out in the presence and the absence of HBAg, according to the methods previously described[2,9,10]. For testing the effect of RNase, two 1 ml aliquots of each of the RNA preparations were incubated at 37° C for 30 min without and with 100 µg of recrystallised protease-free RNase (Calbiochem). Peritoneal cells were added to both aliquots of IRNA and NRNA, incubated at 37° C for 30 min and tested as above for migration. The results of the migration indices are depicted in Table 1.

It was clearly and repeatedly established that IRNA induced the capacity for inhibition of migration of the peritoneal macrophages in the presence of HBAg. The NRNA failed to induce inhibition of migration in the presence of HBAg. The cells treated with NRNA, however, showed inhibition of migration in the presence of PPD which is consistent with the fact that the donors of NRNA were immunised with CFA. The migration of macrophages in the absence of HBAg was unaffected by the treatment of cells with IRNA or NRNA. It was further established that the ability of IRNA to transfer cellular immunity was destroyed by treatment with RNase. Therefore, it may be concluded that experimental transfer of cell-mediated immunity to HBAg with IRNA is a specific phenomenon induced by nondialysable RNA extracts of lymphoid organs of guinea pigs immunised against HBAg. The mechanism of this induction and the molecular characterisation of RNA involved in this transfer remain to be studied.

This work was supported by the Blood Resource Branch of the National Heart and Lung Institute. We thank Barbara Levinson for her assistance in RNA preparations.

Addendum: Because 'immune' RNA-mediated transfer of delayed skin reactivity to tuberculin, varidase and monilia antigens has been accomplished by injection of RNA[11], our results have prospects of clinical application for systemic transfer of immunity to HBAg in man.

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Origin of Unreduced Embryo Sacs in Diploid Potatoes

THERE are clear advantages in carrying out potato breeding at the diploid level, but it is likely that the expression of maximum productivity occurs at the tetraploid level[1]. One way in which diploids can be brought to the tetraploid level is by 2x–4x or 4x–2x crosses. It has long been known that a large proportion of the progeny of such crosses is tetraploid[2,3], and it is generally recognised that unreduced gametes with the somatic chromosome number (2*n*) are produced by the diploid parent and these, with normal gametes (*n*) of the tetraploid parent, give tetraploid offspring.

Particular interest has been shown in such crosses with reports that unreduced pollen is frequently formed by the occurrence of parallel (fused) spindles at the second meiotic division, with the subsequent development of only a single cleavage furrow and dyad production[4,7]. This is genetically equivalent to first meiotic division restitution (FDR) (ref. 8) and all loci from the centromere to the first crossover on each chromosome maintain the same constitution in the gamete as in the diploid parent. There are, however, alternative ways in which unreduced male gametes can arise[8,9]. These, which are genetically equivalent to second meiotic division restitution (SDR) (ref. 8), are characterised by an effective first division chromosome separation and nondisjunction of the products of the second division. The unreduced gametes produced are, in consequence, highly homozygous.

The cytological events leading to unreduced embryo sacs have not been elucidated and it is not known whether their formation can occur by FDR. We report genetic investigations which give some insight into the process.

Table 1 Ploidy and virus X reactions of progeny from 2x–4x crosses

Cross	♀ *Rxrx*	♂ *rx*^{4}	Total progeny	Ploidy 3x	Ploidy 4x	4x Progeny Resistant	4x Progeny Susceptible
a	H8750	Rheinhort	3	0	3	3	0
b	H8513	58.16/2	12	3	9	7	1*
c	H1890	Rheinhort	5	1	4	3	1

* One 4x seedling not tested.

Three diploid clones, H8750, H1890 and H8513, which had quite different breeding histories but were all heterozygous for gene *Rx* from *Solanum acaule*[12] (gene Rx_{acl})[13] which confers extreme resistance to virus X, were used as female parents in 2x–4x (*Rxrx–rxrxrxrx*) crosses. The crosses made, and details of the progeny obtained, are given in Table 1. Ploidy level was determined by root-tip chromosome counts, and reaction to virus X by sap infection (twice) with back testing to leaves of *Gomphrena globosa*.

Table 2 Crosses made to determine genotypes of tetraploid seedlings with extreme resistance to virus X

Cross *	4x resistant progeny		*rx*^{4}	Family
a	4628	×	62.47/20	72.27
b	4612	×	58.16/2	72.19
b	4613	×	62.47/20	72.1, 73.46, 72.21
b	4613	×	Rheinhort	72.20
b	4615	×	62.47/20	70.102
b	4615	×	Hansa	72.22, 73.42
b	4615	×	Rheinhort	72.23
b	4615	×	Grata	73.41
b	4617	×	62.47/20	72.25
b	4617	×	Hansa	73.43
c	4668	×	Record	70.9, 70.10
c	4668	×	Hydra	70.103

* 2x–4x cross: see Table 1.

As expected, most of the progeny were tetraploid (80%) and 86.7% of these proved to have extreme resistance. To ascertain whether the resistant progeny were simplex or duplex, further crosses were made with susceptible tetraploids (Table 2). The crosses involved six of the resistant seedlings from 2x–4x crosses and each was crossed with one or more different tetraploids. Particular crosses were repeated in a number of cases. The resulting families of progeny were tested for virus X reaction with the results shown in Table 3.

Only families 72.1 and 72.25 showed significant deviations from a 1:1 (or 0.87:1 with random chromatid segregation) ratio expected for simplex resistant seedlings. In the former case, further identical crosses confirmed a 1:1 ratio, and in the latter case an alternative cross with Hansa (family 73.43) gave a 1:1 ratio. In general, the data gave a better fit to a ratio based on random chromosome assortment (1:1) than random chromatid assortment (0.87:1). The results clearly show that all six resistant seedlings were simplex, and that 2*n* gametes involved in the crosses producing these seedlings had the same genetic constitution as the parents with respect to this locus.

SDR, in the absence of crossover between the centromere and the *Rx* locus, would give gametes homozygous at this locus. Tetraploid progeny might be expected to segregate 1:1 (resistant:susceptible) with resistant progeny duplex for *Rx*. These duplex progeny would segregate 5:1 in subsequent crosses with susceptible tetraploids. The evidence suggests that unreduced embryo-sac formation was not by SDR since it is unlikely that all the resistant progeny tested had resulted from crossing over between the centromere and the *Rx* locus. The 13:2 (resistant:susceptible) ratio is indicative of FDR which results in simplex resistant tetraploids. Crossing over could account for the two susceptible tetraploids obtained since *rxrx*, *Rxrx* and *RxRx* (1:2:1) gametes should originate in this way. That duplex resistant progeny formed by the involvement of *RxRx* gametes were not found was probably due to the small number of resistant progeny examined. The alternative theory of Koopmans and Van der Burg[14], that mitotic doubling of the reduced chromosome number in the egg nucleus of a diploid occurs when pollen of a tetraploid enters the embryo sac, can be excluded since tetraploid progeny would all be nulliplex or duplex.

Table 3 Segregation in crosses made to determine genotypes of tetraploid seedlings with extreme resistance to virus X

Family *	Progeny		Probability of a larger value of χ^2	
	Resistant	Susceptible	1 : 1†	0.87 : 1 ‡
72.27	9	11	0.75–0.50	0.90–0.75
72.19	56	45	0.25–0.10	0.10–0.05
72.1	26	10	< 0.005	< 0.005
73.46	22	24	0.75–0.50	0.90–0.75
72.21	14	12	0.75–0.50	0.50–0.25
72.20	10	9	0.75–0.50	0.75–0.50
70.102	26	19	0.50–0.25	0.25–0.10
72.22	7	12	0.25–0.10	0.50–0.25
73.42	21	16	0.50–0.25	0.25–0.10
72.23	29	26	0.75–0.50	0.50–0.25
73.41	81	77	0.75–0.50	0.25–0.10
72.25	23	11	0.05–0.025	< 0.005
73.43	27	28	0.90–0.75	0.75–0.50
70.9	52	52	1.00	0.50–0.25
70.10	57	71	0.25–0.10	0.75–0.50
70.103	8	7	0.90–0.75	0.75–0.50
	468	430	0.25–0.10	< 0.005

* See Table 2.
† $Rxrx^3$–rx^4 assuming random chromosome assortment.
‡ $Rxrx^3$–rx^4 assuming random chromatid assortment.

The potential importance in breeding of the use of unreduced gametes fomed by FDR lies in the possibility offered of preserving beneficial intra-locus and inter-locus interactions present in the 2x genotype and transferring them, more or less intact, to the 4x progeny[5]. The use of such intact chromosome sets should, Chase has suggested[1], maximise heterotic potential. Striking heterotic responses and an unusually high degree of within-family uniformity in field characteristics has been observed in 4x–2x progenies[4–6] but not in 2x–4x[4,10,11], and it has been suggested that this reflects fundamental differences between the modes of unreduced pollen and embryo-sac formation. Our own field observations do not suggest fundamental differences in the progenies of 4x–2x and 2x–4x crosses, and this work suggests that unreduced embryo sacs can arise via FDR. In view of the potential value of such inter-ploidy crosses further investigations to determine the relative frequencies of FDR and SDR in the production of both unreduced pollen and embryo sacs would be of the utmost interest.

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Absence of Messenger RNA for Beta Globin Chain in β^0-Thalassaemia

THE β-thalassaemias are a heterogeneous group of hereditary haematological disorders in which there is absent or decreased synthesis of the β globin chain of normal human adult haemoglobin, HbA ($\alpha_2\beta_2$)[1]. When β chain synthesis is totally absent, the condition can be referred to as β^0-thalassaemia, in contrast to β^+-thalassaemia in which some β chain synthesis occurs. The genetics and manifestations of β^0-thalassaemia are rather varied. In the heterozygote, there may be either increased levels of the minor haemoglobin, HbA_2 ($\alpha_2\delta_2$) ('A_2-thalassaemia'), or normal levels of HbA_2 with elevated levels of foetal haemoglobin, HbF ($\alpha_2\gamma_2$), ('$\delta\beta$-thalassaemia' or 'F-thalassaemia'). Homozygous β^0-thalassaemia of the A_2 variety is clinically severe, and has been described mainly in the Ferrara region of northen Italy and in Thailand, although it is found sporadically in other racial groups; the red cells of these individuals contain mainly HbF with variable amounts of HbA_2, but no HbA. Homozygous $\delta\beta$-thalassaemia is extremely rare, having been described in only eight individuals from four different families[1]; it is associated with only mild symptoms; the red cells of these individuals contain 100% HbF, and there is total absence of both HbA and HbA_2.

In the usual form of homozygous thalassaemia (β^+-thalassaemia of the A_2 variety), the decreased synthesis of β chain has been shown to be associated with a deficiency of functional mRNA for β globin chains: mRNA isolated from β-thalassaemic reticulocytes (and marrow cells), when translated in heterologous cell-free systems, programmes the synthesis of much less β than α chain of HbA[2–7]. Furthermore, the β chain mRNA has been shown to be truly deficient (quantitatively) in these patients: hybridisation of thalassaemic mRNA with synthetic DNA (cDNA) complementary to α or β globin mRNA reveals the presence of much less β mRNA than α mRNA[8,9]. In β^+-thalassaemia, therefore, there is no evidence for the presence of normal amounts of a functionally and/or structurally abnormal β chain mRNA unable to participate in protein synthesis. It has been proposed that the β^0-thalassaemia of the Ferrara region may have a different molecular basis: experiments have been performed which suggest the presence, in this syndrome, of substantial amounts of β chain mRNA. When ribosomes from reticulocytes of patients with homozygous Ferrara thalassaemia are incubated in a cell-free system in the presence of supernatant fraction of non-thalassaemic reticulocytes from persons with HbA or HbS, some β^A (but no β^S) chain is apparently synthesised[10,11].

We have now examined by the method of DNA-RNA reannealing or hybridisation, mRNA from two unrelated patients with homozygous β^0-thalassaemia of the A_2 variety, and pooled reticulocyte RNA from three siblings with homozygous $\delta\beta$-thalassaemia. The patients were of southern Italian ancestry and not from Ferrara; the $\delta\beta$-thalassaemics were Sicilian and have been previously reported[12]. Our results indicate a virtual absence of β chain mRNA in reticulocytes of these patients.

A major difficulty in these studies resulted from the presence, in β-thalassaemic red cells, of mRNA for the γ chain of HbF, and the cross homology which evidently must exist in the mRNA sequence coding for the human γ and β globin chains, which differ only in thirty-nine of 146 amino acids.

Hybridisation of globin mRNA to cDNA under non-stringent conditions, or hybridisation of human mRNA to cDNA probes synthesised from non-human globin mRNA as template, can lead to an incorrect assessment of the true amount of β chain mRNA present in a given preparation because of the formation of γ mRNA-β cDNA hybrids[8,9]. We have shown, however, that hybridisation of human globin mRNA with a cDNA probe specific for human β chain mRNA, under stringent conditions, permits the assay of the amount of β chain mRNA present in a given RNA sample without interference from the γ chain mRNA present in that sample[8]. The human β cDNA probe was synthesised from α-thalassaemia mRNA which was shown to contain $\alpha:\beta$ sequences in a ratio of 1:6[8]; and the conditions required to eliminate γ mRNA-β cDNA hybrid formation was to increase hybridisation temperature from 65° C to 78° C[8].

Our initial experiment involved the use of a labelled cDNA probe, synthesised from total human (non-thalassaemic) globin mRNA. This probe apparently consisted of α and β chain sequences in a ratio of approximately 1 to 1. Hybridisation was performed for 40 h at 78° C in 0.2 M sodium phosphate buffer, *p*H 6.8, and 0.5% SDS. The amount (%) of the input cDNA which formed a hybrid, with the mRNA, was then determined by the digestion of the sample with *Aspergillus* S1 nuclease as previously described[8].

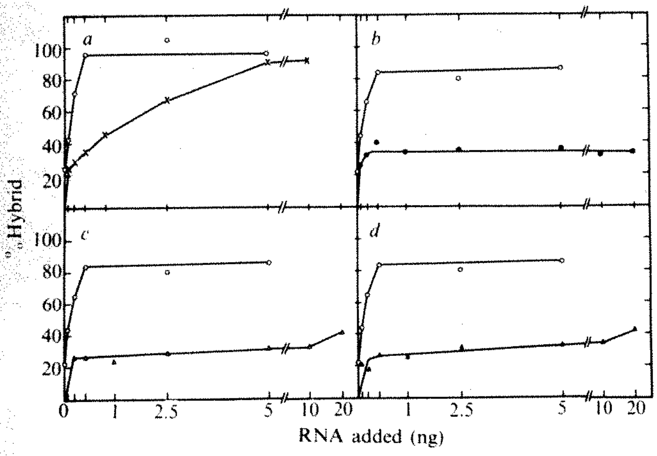

Fig. 1 Hybridisation of human reticulocyte RNA to total human globin cDNA. The cDNA was prepared as previously described[8], but in the presence of ^{3}H-dCTP, 25.5 Ci mmol^{-1}, and non-thalassaemic human globin mRNA. The cDNA input was 220 to 300 TCA-precipitable c.p.m., per reaction; the blanks, after digestion with S1 nuclease, contained 20 to 30 c.p.m. The globin mRNA was prepared from total reticulocyte lysates as previously described[3] and, except for the thalassaemic mRNA in panels *b* and *d*, consisted of the RNA sedimenting between 4S and 18S after sucrose gradient fractionation[3,6]. The thalassaemic RNA in panel *b* consisted of total unfractionated RNA, and in panel *d*, the gradient fractionated RNA sedimenting faster than 4S and including the 28S RNA. The indicated amount of RNA refers to the amount of added 10S globin mRNA as determined by acrylamide gel electrophoresis of the RNA samples or by assuming 10S RNA to be 1% of total cellular RNA. Hybridisation was for 40 h at 78°–80° C, and % hybridisation was determined as previously described[8]. *a*, *b*, *c*, *d*, ○——○, non-thalassaemic human globin mRNA. *a*, ×——×, β^+-thalassaemic mRNA (same patient as in ref. 8). *b*, ●——●, $\delta\beta$-thalassaemia RNA. *c*, △——△, β^o-thalassaemia (A_2 type): patient 1. *d*, ▲——▲, β^o-thalassaemia (A_2 type): patient 2.

The results are shown in Fig. 1, where percentage of hybrid formation is plotted on a linear scale, with respect to increasing amounts of added 10S RNA to each sample. RNA derived from reticulocytes of a non-thalassaemic patient showed a saturation curve with an initial saturation value of 95%, achieved at a very low RNA input (0.5 ng) (Fig. 1*a*). This result indicates that both α and β chain sequences were present in the sample in reasonably high concentration. A sample of RNA derived from reticulocytes of a patient suffering from β^+-thalassaemia also reached a final saturation of 90–95% but two transitions are observed in the saturation curve. The initial slope, which reached a value of approximately 40–45% hybridisation, probably represents mainly hybridisation of the α chain sequences present in the sample, and it was achieved at a relatively low RNA input (0.5–1.0 ng) [Fig. 1*a*]. The second transition, then, would represent hybridisation of the reduced amount of β chain mRNA present in this sample: 90–95% hybridisation was finally achieved at a RNA input of 5–10 ng.

The hybridisation of RNA derived from patients with homozygous $\delta\beta$-thalassaemia (Fig. 1*b*) showed a first transition identical to the transition shown by the β^+-thalassaemic patient: approximately half as much hybridisation as the control (non-thalassaemic RNA) was achieved with 0.5 ng of RNA. No second transition was, however, observed: even after the addition of forty times more RNA (20 ng), there was roughly only half as much hybridisation as achieved by the control RNA, at saturation. This result strongly suggests that some of the sequences present in the normal reticulocytes were absent in the reticulocytes derived from the patients with $\delta\beta$-thalassaemia. RNA derived from reticulocytes of two patients with homozygous β^o-thalassaemia, of the A_2 variety, was also assayed (Fig. 1*c* and *d*). The saturation curves are similar to the curve observed for the RNA derived from the $\delta\beta$-thalassaemia reticulocyte RNA except that in both cases there is a suggestion of the start of a second transition at the highest RNA input (20 ng). These results also indicate that some mRNA sequences present in normal reticulocyte RNA were absent or present in extremely low amounts in the reticulocyte RNA of these patients with β°-thalassaemia.

To assay specifically for β chain mRNA in the RNA samples of these patients, experiments similar to those shown in Fig. 1 were performed, but using a probe specific for human β chain mRNA:cDNA transcribed from α-thalassaemia mRNA[8]. Hybridisation was again performed under the stringent conditions (78–80° C) which eliminated cross-hybridisation between γ and β chain sequences. The results of these experiments are shown in Fig. 2. The non-thalassaemic and β^+-thalassaemic RNA (Fig. 2*a*) yielded saturation curves similar to those obtained in Fig. 1*a*, except that the β^+-thalassaemic RNA showed much less hybridisation at low RNA input and achieves saturation only at a higher RNA input than previously: the deficiency of β mRNA is even more obvious than in Fig. 1*a*. In Fig. 2*b* it can be clearly seen that β chain sequences were totally absent in the RNA derived from reticulocytes of the patients suffering from homozygous $\delta\beta$-thalassaemia: at all mRNA concentrations tested ranging from 0.25 to 100 ng, there was only a baseline level of hybridisation (15%), consistent with the estimated 15% 'contamination' of the β mRNA (α-thalassaemia mRNA) with α mRNA sequences[8]. Hybridisation of mRNA derived from reticulocytes of the two patients with β^o-thalassaemia of the A_2 variety also shows virtual absence of β chain mRNA sequences (Fig. 2*c* and *d*).

A small amount of hybridisation above the 15% baseline level was observed at very high RNA inputs of 50 to 100 ng in A_2-β°-thalassaemia (Fig. 2*c* and *d*). We suggest that this hybridisation may represent hybridisation of trace amounts of of δ chain mRNA sequences (of HbA_2) with the β chain

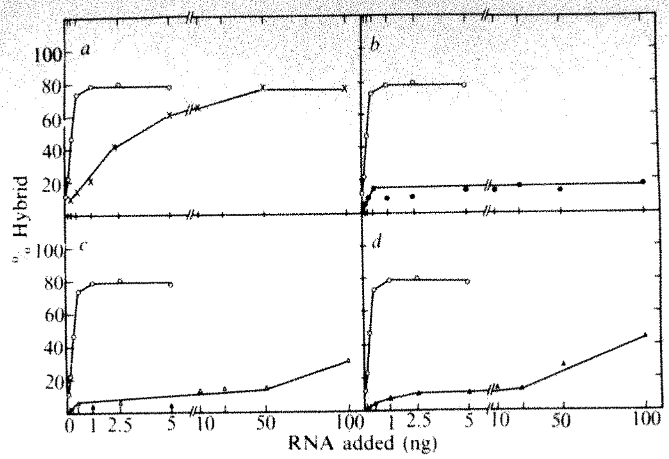

Fig. 2 Hybridisation of human reticulocyte RNA to human β cDNA. The cDNA was synthesised in the presence of ^{3}H-dCTP (25.5 Ci mmol^{-1}) and human β-chain-specific (α-thalassaemic) globin mRNA[8]. Hybridisation was for 40 h at 78°–80° C. The input cDNA was 200–500 c.p.m. and the digested blank had 20–30 c.p.m. The RNAs and their symbols are the same as in Fig. 1.

cDNA probe; the quantity involved was less than 0.5% of the RNA which hybridised in Fig. 1*c* and *d*. The homology between δ and β chains, which differ in only ten of 146 amino acids, is greater than that between γ and β chains and, therefore, cross hybridisation would be expected even under the stringent conditions used. It is not possible to obtain δ chain mRNA in sufficient amounts and purity for the synthesis of δ specific cDNA probes to test this hypothesis. Although it cannot be conclusively established that the small amount of hybridisation observed at high RNA inputs represented δ chain mRNA rather than trace amounts of true β chain mRNA, it is nonetheless clear that very little (if any) β chain mRNA was present in the reticulocyte RNA of our patients with β°-thalassaemia. Certainly, these patients of southern Italian ancestry did not have the defect which has been proposed for the Ferrara type of β°-thalassaemia. Finally, it should be noted that all the RNA samples tested were derived from total reticulocyte lysates and not from polysomes: our experiments, therefore, ruled out the existence in these thalassaemic reticulocytes of β chain mRNA which is unable to enter polysomes and participate in protein synthesis. Furthermore, the use of unfractionated RNA and RNA which includes all of the RNA between 4S and 28S, ruled out the unlikely existence of a high molecular weight abnormal mRNA which would otherwise be missed by using only RNA from the 9–10S region of the sucrose gradient.

In conclusion, these results suggest that a feasible hypothesis to explain the molecular defect, in certain forms of β°-thalassaemia, is a deletion of the DNA sequences coding for the β chain mRNA (and, in addition, for δ chain mRNA in δβ-thalassaemia). The ability to hybridise β chain cDNA sequences, specifically, in the presence of vast excesses of cellular DNA from patients with β°-thalassaemia, will permit the testing of this hypothesis.

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Renal Concentration of Citrate as a Negative Modulator of Diuretic Response to Mercurials

In several mammalian species, including man, the potency of organomercurial diuretics depends on the acid-base status. Acidifying salts (for example, ammonium chloride) markedly enhance the diuretic response to mersalyl[1]. To account for this, Weiner *et al.*[2] proposed that (*a*) hydrogen ions facilitate the cleavage of the carbon-mercury bond of organomercurials and (*b*) the diuretic response is elicited by the interaction of mercuric ions with renal diuretic receptors. The authors based their conclusions on the excellent correlation between the liberation of mercuric ions from a given organomercurial under acidotic conditions *in vitro* and its diuretic potency in dogs *in vivo*. Recently we reported that rapid alkalinisation of urine brought about by treatment of dogs with acetazolamide decreases the diuretic response to inorganic mercury complexed with cysteine[3]. This observation led us to postulate that alterations in acid-base balance not only modify the rate of cleavage of the carbon-mercury bond in organo-mercurials but also must control the diuretic response to mercuric ions. Our findings are compatible with the following hypothetical chain of events. Alkalosis increases and acidosis decreases the renal concentrations of an en-

Table 1 Urinary *p*H and tissue citrate concentrations in dogs

Before or after treatment	Acid	Treatment Base	NaFAc
		Urinary *p*H	
Before	6.25	7.05	6.91
After	5.35	7.89	6.99
		Urinary excretion of citrate (μmol kg^{-1} min^{-1})	
Before	0.0024	0.0017	0.0025
After	0.0030	0.068	0.47
		Plasma citrate (μmol ml^{-1})	
Before	0.120	0.152	0.115
After	0.052	0.152	0.249
		Citrate in renal cortex (μmol g^{-1} fresh weight)	
After	0.048	0.131	2.61
		Citrate in renal medulla (μmol g^{-1} fresh weight)	
After	0.069	0.180	0.682

The animals were killed after treatment for 2 h with hydrochloric acid or sodium hydroxide (priming dose of 1,000 μmol kg^{-1} intravenously and sustaining infusion of 1,000 μmol kg^{-1} h^{-1}) or with sodium fluoroacetate (Na-FAc, 0.5 mg kg^{-1} intravenously). The values represent averages for two dogs in each treatment group.

dogenous metabolite which is capable of complexing mercury. In the kidneys the partition of mercuric ions between the diuretic receptor and the hypothetical ligand will then depend on the relative concentration of these two substances and their relative affinity for mercury. Specifically, the ratio of mercury bound to these two sites can be described by the following expression

$$\frac{[\mathrm{Hg{\cdot}L}]}{[\mathrm{Hg{\cdot}R}]} \propto \frac{[\mathrm{L}]{\cdot}K_{\mathrm{L}}^{\mathrm{Hg}}}{[\mathrm{R}]{\cdot}K_{\mathrm{R}}^{\mathrm{Hg}}}$$

where brackets denote the renal concentration, L the hypothetical ligand, R the diuretic receptor, Hg·L and Hg·R the corresponding complexes with mercury, and K^{Hg} the effective stability of mercury complexes with the compound denoted in the subscript. A change in any one of the four factors on the right side of the expression will alter the partition of mercury between these two binding sites. If the concentration of the ligand increases in alkalosis, more mercury will be sequestered by the ligand and, as a consequence, a smaller number of mercuric ions would be free to react with renal diuretic receptors. The predicted result will be a decreased diuretic potency of inorganic mercury. Under acidotic conditions when the renal level of the ligand is low, the binding of mercury to the diuretic receptor would not be opposed to the same extent. Therefore the full diuretic response will ensue. The hypothesis does not require that changes in acid-base status alter either the total concentration of mercury in renal tissue or the rate of urinary excretion of mercury. In any case a large fraction of renal mercury is bound to non-specific sulphydryl groups. Many sulphydryl groups of proteins may serve as scavengers for mercury and thus do not participate in the diuretic response[1,4].

Citrate anion most fully meets all the requirements of the hypothetical ligand. First, citrate forms stable chelates with a host of divalent cations[5]. The stability of the mercury-citrate chelate has not been determined. The absolute value of the stability constant of the mercury-citrate complex is, however, not required to test the hypothesis. The main postulate—that a change in citrate concentration produces a shift in the intrarenal distribution of mercury—holds true independent of the affinities of the diuretic receptor and citrate for mercury. Even if the stabilities of mercury complexes with the diuretic receptor and with citrate were known and if the former were found to be 100 times greater than the latter, citrate may still compete effectively with the receptor for mercury if its concentration is about 100 times greater than that of the receptor. In regard to the second postulate, changes in acid-base status produce the requisite changes in renal level of citrate. In the kidneys of rats, citrate levels are elevated under alkalotic and decreased under acidotic conditions when compared to control values[6-8].

To test our hypothesis we chose conditions which would alter the renal concentration of citrate with or without inducing concomitant changes in acid-base status. Two series of experiments were performed on anaesthetised dogs of either sex. Experimental procedures were identical to those described elsewhere[9]. The first series of experiments was intended to determine whether the citrate concentrations in the kidneys are altered in the desired direction in dogs infused with hydrochloric acid or sodium hydroxide for 2 h, or injected with sodium fluoroacetate. Citrate in urine, kidneys and plasma was determined by the method of McArdle[10]. The data of Table 1 show that the conditions are met and, in particular, that fluoroacetate elevates renal concentrations of citrate without changing urinary *p*H.

In the second series of experiments, dogs were treated as in the first series. At the end of the 2 h pretreatment period

Table 2 Glomerular filtration rate and the urinary excretion of electrolytes in dogs

	Acid	Acid and uranyl	Base	NaFAc
		GFR (ml kg^{-1} min^{-1})		
Before Hg	3.88±0.50	4.01 ±0.16	3.60 ±0.61	3.91 ±0.14
Change	−0.50±0.25	−0.95 ±0.10	−1.04*±0.19	−1.36 ±0.10
		Na excretion (μmol kg^{-1} min^{-1})		
Before Hg	25.4 ±7.4	18.7 ±4.3	26.8± 4.1	39.2±6.8
Change	71.3 ±10.5	50.3 ±2.3	13.0±11.3	2.5±9.9
		Chloride excretion (μmol kg^{-1} min^{-1})		
Before Hg	26.3 ± 7.1	20.0 ±4.1	14.3±2.1	35.5±6.8
Change	63.8*±12.0	51.2†±3.5	11.5±9.9	2.5±8.3
		K excretion (μmol kg^{-1} min^{-1})		
Before Hg	6.01 ±0.40	4.11±0.54	5.57±0.72	7.86±0.66
Change	0.81†±0.08	0.15±0.36	−1.85±0.70	−4.85*±0.99

Dogs were pretreated with hydrochloric acid, sodium hydroxide or sodium fluoroacetate as described in Table 1, and subsequently injected with mercury-cysteine complex [5 μmol $Hg(NO_3)_2$+25 μmol cysteine kg^{-1} intravenously]. Uranyl benzoate (3.5 μmol kg^{-1} intravenously) was injected just before mercury in one group of dogs pretreated with hydrochloric acid. GFR was measured as the clearance of inulin. Changes were during the second hour after the injection of mercury. Four dogs in each treatment group. Data are given as mean ±s.e.
* $P<0.05$. † $P<0.01$; that the changes differ from zero.

(see above) mercury-cysteine complex was injected [5 μmol $Hg(NO_3)_2$+25 μmol cysteine per kg body weight intravenously]. To produce a decrease in the glomerular filtration rate (GFR) comparable with that expected in the two other groups, uranyl benzoate (3.5 μmol kg intravenously) was administered to groups of acidotic dogs just before the injection of mercury. Except for the effect on GFR, uranyl does not interfere with mercurial diuresis[11]. The excretion of electrolytes in urine was followed for 2 additional hours. During the second hour a marked diuretic response to mercury-cysteine complex was observed in all acidotic dogs but not in the four alkalotic animals or in those treated with fluoroacetate (Table 2). There was a slight decrease in GFR in dogs pretreated with acid alone and a larger decrease in acidotic dogs treated with uranyl benzoate, in alkalotic dogs, and in fluoroacetate pretreated dogs. It is clear, therefore that a drop in GFR though reducing the maximal diuretic response to mercury cannot account for the total absence of diuresis in alkalotic dogs or in those pretreated with fluoroacetate. The results are thus in agreement with and furnish experimental support for our hypothesis. The following additional observations were made. (*a*) Urinary excretion of radiomercury, administered along with mercury-cysteine complex, was similar in all four groups. (*b*) The corollary to the hypothesis, namely, that fluoroacetate should not interfere with the diuretic response to nonmercurial diuretics was demonstrable. The diuretic response to ethacrynic acid (5 mg kg^{-1} intravenously; two dogs) and furosemide (10 mg kg^{-1} intravenously; two dogs) was not inhibited by the pretreatment with fluoroacetate. In these dogs the increment in the urinary excretion of sodium amounted to 40 to 50 μmol kg^{-1} min^{-1}. The diuretic response to ethacrynic acid is especially noteworthy since mercurials and ethacrynic acid may have the same mechanism of action at the cellular level[9]. If renal citrate prevents mercurial diuresis by chelating mercuric ions then the observed diuretic response to ethacrynic acid agrees with our hypothesis since a corresponding reaction between citrate and the anion of ethacrynic acid cannot occur. (*c*) Renal concentrations of citrate in normal rats, as reported by Crawford[6] and others[7,8], are higher than in alkalotic dogs in our experiments. This fact is in accordance with the known low potency of mercurial diuretics in rats. Our preliminary study in this species, in addition to confirming the older data, showed a four-fold increase of citrate concentrations in the kidneys, but not in the liver, of rats treated with mercury-cysteine complex. Mercurial diuretics may, therefore, be 'self-inhibitory' in this species.

Although all these findings support our hypothesis, the possibility that increased renal concentrations of citrate alter the response to mercurial diuretics in some way other than by chelation of mercuric ions cannot be excluded at the present time.

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Autoradiography of Biological Tissues in the Scanning Electron Microscope

AUTORADIOGRAPHY combined with light or transmission electron microscopy has become a very valuable tool in biological research[1,2]. Light microscope (LM) autoradiographic resolution is, however, limited by the resolving power of the optical system. A ten to twenty times improvement in autoradiographic resolution (up to 50 to 70 nm) can be obtained in the transmission electron microscope (TEM) but a number of physical factors limit the system[1,2] including the size and the sensitivity of the photographic emulsion silver halide crystal and the grain-size. Further, ultrathin TEM sections limit detection of radioactive material in labelled specimens by the very small fraction of total incorporated radioactivity present in such sections. This leads to a practical limitation of TEM autoradiography for, in addition to the already time-consuming preparation of ultrathin sections, high levels of radioactive labelling and long exposure times (about ten times that of LM autoradiography) must in general be used to compensate for the low efficiency of the procedure.

It is of interest, therefore, to consider the possibility of combining autoradiography with scanning electron microscopy (SEM)[3] as the resolution of the SEM (7 to 20 nm), although inferior to that of the TEM, is within the range attained in TEM autoradiography. A number of advantages are suggested by the use of such a system including the ease and rapidity of specimen preparation, the larger sample areas and the possible improvement of autoradiographic efficiency using smaller crystal emulsions and finer compact grains—detection of developed grains being helped by the depth of field and analytical facilities of the SEM.

In this preliminary investigation on the biological application of SEM autoradiography bladder explants from 6-month-old C57^{at}Icrf mice were cultured *in vitro*[4] for 4 d on medium containing ^{3}H-thymidine (1 μCi ml^{-1}, 5 Ci $mmol^{-1}$) and 4 h before fixation colchicine (0.1 mg ml^{-1}) was added to the medium. The tissues were fixed in Bouin's fluid, embedded in paraffin wax and 5 μ thick serial sections prepared. These were mounted on carbon-coated[5] and on uncoated 13 mm Chance coverslips. Control non-radioactive explants were processed in parallel with the radioactive tissues.

To facilitate subsequent manipulation of the coverslips, these were provisionally mounted with DPX, section-side uppermost, on glass slides. The sections were dipped in a 1 : 1 Ilford L4 nuclear track emulsion and exposed for 1 week at 4° C (ref. 6). The autoradiographs were developed with Kodak D19 for 5 min at 18° C and fixed for 10 min in Ilford Hypam. These were either left unstained and air-dried for scanning electron microscopy or post-stained with Mayers' haematoxylin-eosin stain and mounted for light microscope observations. To determine if the surface detail of the emulsion-covered sections could be enhanced for scanning electron microscopy the gelatin of the emulsion was removed in a number of sections by treatment with 0.05 N sodium hydroxide for 20 min at 18° C (ref. 7). Specimens mounted on carbon-coated coverslips were viewed initially in the SEM without any metal coating. Subsequently, an approximately 20 nm electrically conducting layer of aluminium or gold was applied to both carbon-coated and uncoated coverslips. The specimens were examined with a Cambridge Scientific Instruments Stereoscan Mk IIa scanning electron microscope using an accelerating voltage of 30 kV and a specimen tilt angle of 45° unless otherwise noted. With an Ortec Si(Li) solid state energy dispersive detector coupled with a Northern Scientific Econ II multi-channel analyser, X-ray elemental and distribution analyses of silver were mapped using the $L\alpha$ line. The X-ray analyses were performed at an accelerating voltage of 20 kV, beam current of 120 μA, and a filament current of 2.5 A. Lens settings were at 0.42 A (top), 0.37 A (middle), and 0.60 A (final lens). X-ray count times varied from 3,000 to 5,000 s.

Light miscroscope observations showed concentrations of black silver grains over the cell nucleus, the radioactive material being present mainly in the epithelial cells of the bladder cultures with very little uptake of label occurring in the stromal tissues. Specificity of labelling was demonstrated by a comparison with the control, non-radioactive bladder sections which showed only a very low random background distribution of grains. A specific distribution of grain-like structures, similar to that seen under the light microscope, was found in the scanning electron autoradiographs (Fig. 1*a*). This distribution could be directly correlated with the light microscope autoradiograph by light microscope observations of the SEM specimen (Fig. 1*b*). Because of their higher back-scattering coefficient, the grain structures were easily identified in the uncoated sections on carbon-coated glass (Fig. 1*a*). Although at low magnifications charging problems were unimportant, it was necessary to metal coat the specimens to make detailed observations of the grain structures at higher magnifications. The metal coating had the effect of reducing the difference in back-scattering coefficients between the silver and the matrix. Structural details of the section were found to be enhanced following sodium hydroxide treatment (Fig. 2).

Irregular, ribbon-like filaments, thickened at discrete points along the surface extended from crystalline structures which, in general, demonstrated a cubic shape (Fig. 3). This morphological picture correlates closely with published photomicrographs in transmission electron microscope studies on silver filament formation[8-10] and the grain structures observed in the scanning electron micrographs may be interpreted, therefore, as developed grains consisting of metallic silver ribbons extending from cubic silver halide crystals. It was sometimes difficult to see the characteristic filamentous form of the D19 developed L4 emulsion grains[11], but structural visualisation was markedly improved by alteration of the tilt angle of observation from 45° through 15° or 0°. The distribution of silver grains was

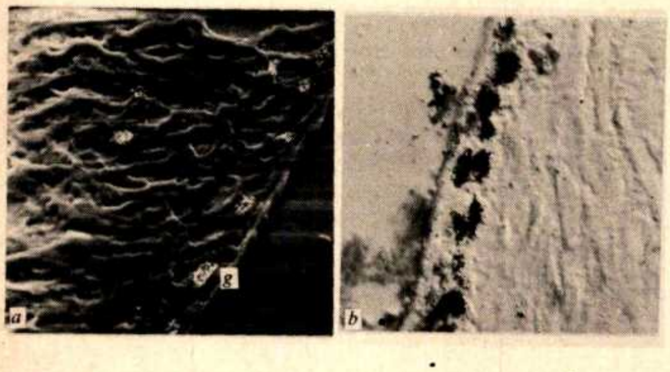

Fig. 1 Autoradiographs of bladder showing concentrations of silver grains over nuclei of cells. *a*, Uncoated SEM preparation mounted on carbon-coated coverslip. Grains, *g*, are easily identified by their higher back-scattering coefficient (45° tilt) (×213); *b*, light micrograph of (*a*). (×135.)

easily determined using back-scattered electron imaging[12] in the scanning electron microscope. This was found to provide a system analogous to darkfield illumination in LM autoradiography[1] in that the silver grains, because of their higher atomic number, have a back-scattering coefficient more than four times that of the tissue material[13], and appeared as bright structures on a dark background (Fig. 4*a* and *b*). The brightness and structural definition of the silver grains were dependent on the angle of tilt of the specimen to the incident electron beam (Fig. 4*a* and *b*) and the highly electron reflective silver halide crystals could only be readily distinguished from the less electron-reflective silver filaments at low angles

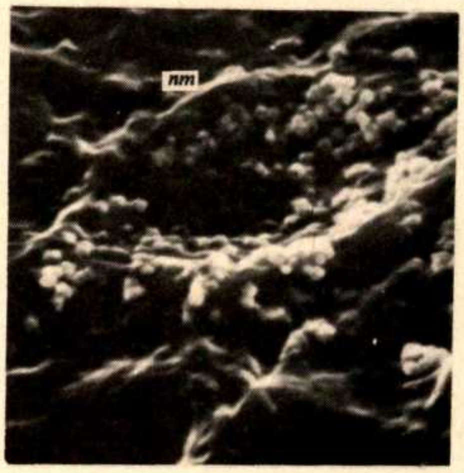

Fig. 2 SEM autoradiograph of sodium hydroxide treated bladder section (Au coated) showing grain concentration in nucleus and resolution of nuclear membranes (*nm*) (45° tilt) (×11,400).

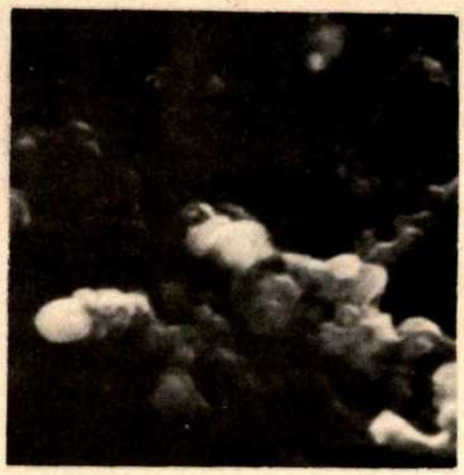

Fig. 3 SEM micrograph of silver grains showing irregular ribbon-like filaments extending from highly electron-reflective cubic Ag halide crystals (15° tilt) (×12,000).

of tilt (0° to 15°). The background grains, found randomly scattered over the sections and glass coverslips were noticeably smaller that the grains produced over sites of radioactivity and corresponded to previous LM observations[1].

Further evidence of the nature of the grain structures was provided by X-ray elemental analysis for silver over labelled and unlabelled areas of the tissue and by distribution mapping of the Ag X rays. Spot analyses on individual grains and small area X-ray analyses over labelled bladder epithelium showed well-defined Ag $L\alpha$ peaks with peak-to-background

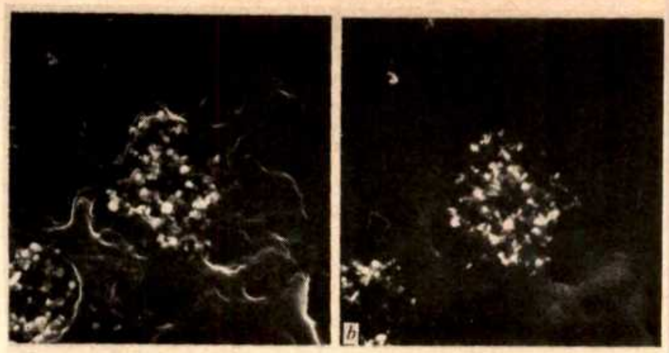

Fig. 4 SEM autoradiograph of bladder epithelial cells (Au coated). *a*, Secondary emissive; *b*, back-scattered electron image analogous with LM darkfield illumination[1] (15° tilt) (×2,130).

ratios of 4:1 and 2:1 respectively, while only a trace of silver was found over unlabelled cells. X-ray distribution mapping of silver using the Lα line showed silver localised in regions of grain concentrations.

Our observations show that autoradiography combined with scanning electron microscopy should provide a technique enabling rapid autoradiographic localisation of various compounds which can be correlated with fine structural detail. The system promises to be of particular value combined with automated image analysis procedures[14] for quantitative autoradiographic data retrieval.

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Lengthening of Illumination Period is a Factor in Averting Diapause

DURING studies of wing length and diapause of *Gerris odontogaster* (Zett.) (Heteroptera, Gerridae), I claimed[1] an intimate correlation between the micropterous and nondiapause conditions and the macropterous and diapause conditions respectively, in pre-overwintering populations. I also summarised the general determination of wing lengths and diapause (Fig. 1), and later a macropter with fully developed eggs was collected from a dimorphic population.

The effect of stationary daylengths, however, was omitted from the figure and although I demonstrated[1] that in a rhythm of 18 h at about 1,000 lux: 6 h at about 80 lux (instead of a planned dark period) both macropter and micropter imagos emerged, the reproductive stage was left implicit in the text. Therefore, my conclusion[1] that a lengthening day averts diapause, has been criticised because of lack of evidence in stationary light conditions[2].

To settle this problem, results of six partly unpublished experiments are now given here (Fig. 2). In experiment *E* some parentals collected on September 14 were first kept at room temperature (about 24° C) in natural photoperiod. No eggs were produced and the individuals became inactive. They were transferred on October 10, to a light:dark photoperiod of 24:0. The first copula was seen on October 30. The rest of the parental batch was taken straight to continuous illumination on September 15. First larvae emerged on October 6, that is, egg laying had begun in September. Therefore the breaking of diapause seems

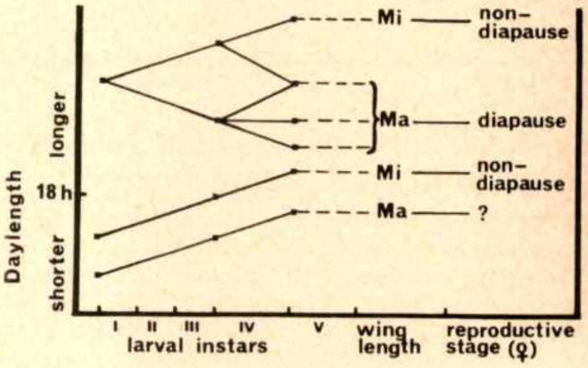

Fig. 1 Determination of wing length and diapause in *Gerris odontogaster* according to ref. 1. The last critical stage is the fourth instar. Mi, Micropter imago; Ma, macropter imago.

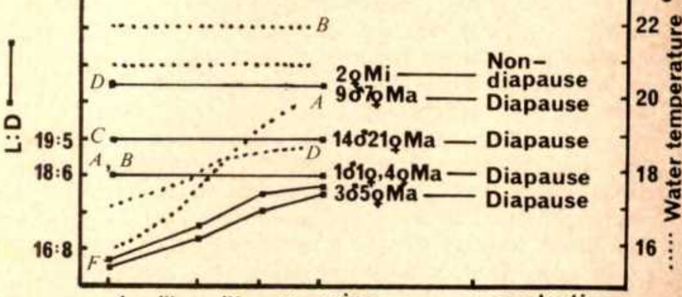

Fig. 2 Reproductive diapause in *Gerris odontogaster* as a response to long, stationary illumination or short, lengthening day rhythm. Light equipment as in ref. 1. The reproductive stage (egg laying) was checked by keeping the adult offspring in cultures from 4 to over 20 weeks and sometimes by dissecting them later. The only offspring to lay eggs were the micropters (*D*), one in the age of ≤15 d, and the other of ≤11 d. Parentals: *A*, F_1 generation macropter from ref. 1; *B*, *C*, *E*, *F*, from the Finnish uniform grid 664 : 28 and, *D*, 671 : 23. In *C*, some few individuals developed through their fifth instar in a long, shortening day rhythm. In experiment *D*, the L : 'D' ratio was 18 h (1,000 lux) : 6 h (80 lux). ..., Water temperature; ■—■, L : D; Mi, micropter; Ma, macropter.

not only to demand cold treatment but also a succeeding period of illumination other than short or shortening.

Summarizing the information in Figs 1 and 2, a long, stationary illumination is usually not enough to avert diapause in *G. odontogaster* if it is a light-dark cycle of 18:6 or 19:5 or continuous illumination, or something in between (the curious 1,000 lux:80 lux rhythm). In some extreme cases of stationary illumination, however, the diapause reaction is ambivalent, guiding some larvae to immediately reproducing imagos and others to diapause. Furthermore, a short day, even if lengthening, causes diapause. Therefore, to effectively avert diapause, a long, lengthening day rhythm is necessary. Remarkably, even under stationary light conditions the correlation between wing length and the reproductive stage is closely maintained, with a preponderance of long wings and diapause.

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Nitrosocarbaryl as a Potent Mutagen of Environmental Significance

THE widespread accumulation of environmental pollutants has led to concern over their long-range effects on living organisms. Although many herbicides, pesticides, and food additives have no deteremental effect on experimental animals, the possible chemical interaction of these compounds with each other or with natural elements of the environment to produce biologically active compounds has rarely been considered. We have found that the combination of a food additive, sodium nitrite, with a commonly used pesticide, carbaryl, results in the formation in acid solution of a new mutagen of considerable potency, nitrosocarbaryl (NC). A study of the reactions leading to formation of mutagens and carcinogens from several types of pesticides and herbicides is reported elsewhere[1]. We present here an examination of the mutagenicity of NC in two bacterial systems, *Escherichia coli* and *Haemophilus influenzae*.

The preparation of a compound assumed to be nitrosocarbaryl has been reported previously in a paper concerned with analysis of trace residues of pesticides[2]. Formation of the nitroso derivative renders such compounds detectable at extremely low concentrations by polarimetric analysis. NC was prepared in quantity from carbaryl according to the following method. Twenty grams of N-methyl-1-naphthylcarbamate (carbaryl; Union Carbide Corporation, New York) was dissolved in 25 ml dimethylsulphoxide, and 10 g of ice was added, together with 20 ml acetic acid and 12 ml hydrochloric acid. Into the thick suspension was stirred 20 g sodium nitrite, the reaction mixture being cooled in an ice bath. The mixture was kept in a freezer for 2 d with occasional stirring, and an orange yellow colour slowly developed. After removal from the freezer, 200 ml ether was added, forming a deep orange upper layer, which was separated from the clear aqueous layer. The ether solution was dried with anhydrous magnesium sulphate, filtered, and evaporated to dryness in a stream of nitrogen at room temperature. The residual solid was dissolved in 30–40 ml ether, which was again evaporated in nitrogen. A first crop of cream yellow crystals was filtered off; these melted at 66°–68° C, and the yield was 7.9 g. A second crop of crystals obtained by evaporating the mother liquid almost to dryness weighed 8.7 g and melted at 63°–65° C. The total yield was 72%. The molar absorptivity of the first crop of crystals was 144 at 402 nm in ethanol, and the absorption spectrum was almost identical with that of N-nitroso-N-methylurethane. The analysis calculated for $C_{12}H_{10}N_2O_3$ was C, 62.60; H, 4.37; N, 12.17; the analysis found was C, 62.60; H, 4.56; N, 11.99. The mass spectrum (70 eV) showed a parent ion at m/e 230 and fragments at m/e 143 (naphthoxyl) and m/e 127 (naphthyl). The compound, N-nitroso-N-methyl-1-naphthylcarbamate (nitrosocarbaryl, NC), was fairly stable in the dry state and after standing in light and air at room temperature showed a drop in absorptivity of less than 10% after 3 days and 20% after 2 weeks.

To characterise its biological activity, the mutagenicity of NC was compared with that of nitrosomethylurethane (NMUr) and N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), the latter being a widely used mutagen in experimental systems. NMUr, a known mutagen and carcinogen[3], is a structural analogue of NC. Decomposition of mutagenic nitroso compounds, thought to be an important factor in their biological activity[4], was found for our compounds to be less than 5% in 1 h in treatment buffer (Tris-maleate, *p*H 6), as followed by ultraviolet absorbance. Since mutation/survival ratios tended to show maxima, we found it useful to compare our compounds on the basis of the relative concentrations required to give similar mutation curves. Survival was measured in each case, as seen in Fig. 1. In each experiment, a standard concentration of MNNG was tested as a positive control, against which the other compounds could be compared. An aliquot of cells was also treated with solvent alone for measurement of the spontaneous mutation rate, which can be used to calculate the induced level of mutation.

In general, the mutation rate increased as a function of

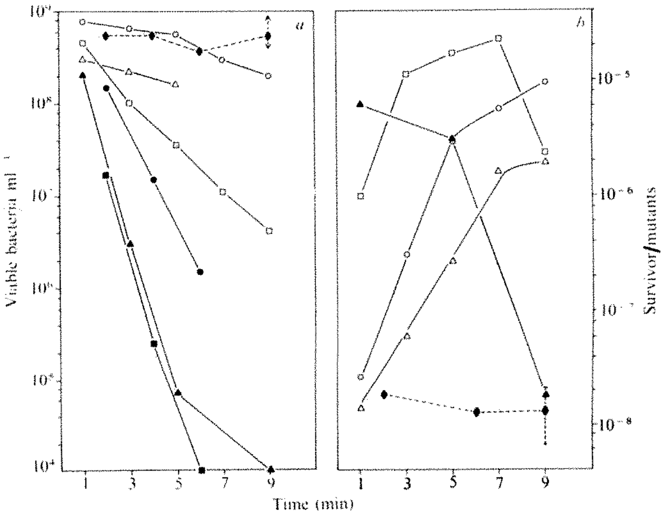

Fig. 1 Kinetics of survival (*a*) and mutation (*b*) of *H. influenzae* after treatment with NC, MNNG, and NMUr. *H. influenzae* (wild type strain Rd or a streptomycin-resistant derivative of Rd) was grown to log phase, pelleted and resuspended in 0.05 M Tris-maleate buffer (*p*H 6) (approximately 6×10^8 cells ml^{-1}), and exposed to the mutagen for a short time. Mutagens were dissolved and further diluted in ethanol, followed by a final 1 : 50 dilution into the bacteria. Treatment was stopped by ten-fold dilution (and further dilution and plating for survival determinations) and subsequent washing by centrifugation and resuspension of the pellet. Treated samples were then resuspended in growth medium and incubated at 37° C to allow expression of mutations. Cultures grown to a standard density were frozen in aliquots and later tested for mutation to novobiocin resistance by plating under novobiocin agar (2.5 μg ml^{-1}), as previously described[5], *a*, Survival after treatment with 0.1 mM NC (■); 0.01 M NC (□); 1.0 mM NMUr (▲); 0.1 mM NMUr (△); 1.0 mM MNNG (●); 0.5 mM MNNG (○) or 0.1 mM carbaryl (◆). *b*, Mutation after treatment with 0.01 mM NC (□); 1.0 mM NMUr (▲); 0.1 mM NMUr (△); 0.5 mM MNNG (○); or 0.1 mM carbaryl (◆). (←--→) indicates area of untreated control. (---) indicates area of spontaneous mutation.

killing up to a loss of about 90% of the cells, after which the observed mutation frequency levelled off and then declined, presumably due to killing of mutants (Fig. 1*a–b*). The dependence of the shape of the curve on mutagen concentration is illustrated by the NMUr results at a ten-fold difference in concentration (Fig. 1*b*). The molar concentration of NC required to induce a mutation frequency of 10^{-5} is less than one-fiftieth the concentration of MNNG required, and less than one-tenth the concentration of NMUr required to induce a similar level of mutation. The precursor, carbaryl, was not mutagenic (Fig. 1*b*).

All three of the compounds tested are N-methyl-N-nitroso compounds. The difference in their potency may be due to differential formation of reactive intermediates within the cell or to differential uptake of the compounds, governed by the structure of the rest of the molecule. The presence of the naphthyl group on NC, which increases its lipid solubility, appears to be a factor in its biological activity.

E. coli strain H/r 30 R requiring arginine was used for measurement of mutation to prototrophy, according to procedures previously described[6]. *E. coli* was found to be less sensitive than *H. influenzae* to both MNNG and NC. NC at 0.1 mM and MNNG at 1.0 mM killed approximately 50% of the *E. coli* cells after a treatment time of 10 min. The induced mutation frequencies (reversion to prototrophy) for these treatments were 3×10^{-4} and 5×10^{-4} per survivor, respectively. Both killing and mutation were less sensitive functions of mutagen concentration and treatment time in *E. coli* than in *H. influenzae*. These results are in agreement with previous studies of MNNG in *E. coli*[7].

Our two test systems were chosen arbitrarily with respect to both organism and genetic loci at which mutations were assayed. Since NC was more active than MNNG in both systems, we conclude that NC is a potent mutagen, which may be of interest to experimentalists studying mutation phenomena. NC is also a possible hazard to man, since its precursors carbaryl and nitrite are both common environmental constituents which are consumed (carbaryl as a crop residue[8] and nitrite as a food additive), and the stomach provides the acidic conditions for its formation[1,9]. This interaction is likely to be biologically significant because N-nitroso compounds are known to act systemically and to be most effective as carcinogens when administered in small doses over long periods of time. No animal species have yet been found to be resistant to the biological effects of N-nitroso compounds[3], and on the basis of the known carcinogenicity and mutagenicity of its close structural analogue NMUr[3], nitrosocarbaryl is likely to be a carcinogen as well as a mutagen.

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Physiological Variation in Circulating B Cell:T Cell Ratio in Man

T LYMPHOCYTES in human peripheral blood may be identified by their ability to form rosettes *in vitro* with untreated sheep erythrocytes[1-7]. This test has been widely applied to detect disturbances in the B:T cell ratio in various disease states[3,5,7-11], there is, however, no published information on the stability or otherwise of the blood B:T cell ratio in health. Such studies are required because it is known that fluctuations occur readily in the numbers and proportions of the different classes of circulating leukocytes[12]. Diurnal variation principally affects the granulocytes but exercise, emotion and the administration of adrenaline are all known to provoke a rapid and substantial lymphocytosis through mobilisation of cells normally sequestered in undetermined sites[12-15]. Here we report investigations on the effect of exercise.

Peripheral blood was obtained from six healthy young laboratory workers. From each sample an aliquot (0.5 ml) was mixed with a chelating agent (sequestrine) for determination of the total white cell count in an electronic counter and for making air-dried smears which were stained by May-Grunwald-Giemsa. The proportion of lymphocytes in the sample was determined by counting at least 500 cells from these smears. The remainder of each sample was defibrinated and lymphocytes were separated from it by centrifugation over a Ficoll/Triosil layer[16].

The sheep-cell rosette technique used in this study has been described in detail elsewhere[17]. Erythrocytes from a selected animal were stored in Alsever's solution at 4° C for up to 2 weeks before use. Triplicate samples of human lymphocytes and sheep red cells were mixed in the presence of 10% foetal calf serum, sedimented at 50 *g* for 5 min and allowed to stand overnight before reading the percentage of rosettes in a total of at least 200 lymphocytes from each tube.

Starting at about 0930 h, each subject undertook 10 min of vigorous exercise (running upstairs). Blood was drawn immediately before beginning the exercise, 5 min after the end of it and 45 min, 2 h and 4 h from the start of the experiment.

In every case there was a marked lymphocytosis after exercise, most of the increase comprising non-rosette-forming cells (presumptive B cells) (Table 1*a*, Fig. 1). By 45 min the total lymphocyte count and the percentage of rosette-forming cells had returned to the pre-exercise level. In the later samples there was a polymorph leukocytosis of extremely variable degree.

The finding that the majority of rapidly mobilised lymphocytes are presumptive B cells is in keeping with other evidence that the influx of cells does not occur through the thoracic duct[15,18] (the lymphocyte content of which comprises mainly T cells[19,20]).

Control experiments, in which blood was drawn at the same time intervals but without the period of strenuous exercise, demonstrated that there is no consistent pattern of diurnal variation in the blood B:T cell ratio (Table 1*b*). Comparing the different samples from each subject, however, some fluctuation in the proportion of rosette-forming cells was observed. The largest range recorded was 11% (59.3 to 70.3%) and the smallest 5.4% (64.0 to 69.4%). We have previously noted a similar variation in the percentage of rosette-forming cells in blood samples collected on dif-

Table 1 Numbers of B and T cells in circulation

	Time (min)	Total WBC mm^{-3}	Total Lymphocytes mm^{-3}	% RFC (range)	Total RFC mm^{-3}	Total non-RFC mm^{-3}
a						
	0	4,088 (1,035)	1,632 (262)	61.8 (53.8 to 63.4)	1,012 (190)	620 (106)
	15	5,547 (1,390)	2,757 (690)	46.4 (41.8 to 50.9)	1,273 (310)	1,484 (432)
	45	4,416 (1,718)	1,693 (312)	60.2 (57.7 to 64.6)	1,017 (200)	675 (140)
	120	5,201 (1,824)	1,889 (386)	56.3 (37.2 to 64.6)	1,050 (260)	840 (323)
	240	5,573 (150)	1,754 (70)	60.6 (52.5 to 65.9)	1,062 (98)	692 (97)
b						
	0	4,024 (654)	1,623 (245)	62.0 (55.4 to 67.6)	1,003 (170)	622 (140)
	15	4,032 (626)	1,628 (318)	62.2 (58.1 to 66.9)	1,004 (139)	624 (182)
	45	4,229 (432)	1,716 (262)	62.9 (59.3 to 69.4)	1,074 (131)	643 (149)
	120	5,056 (1,114)	1,689 (119)	61.8 (53.5 to 70.3)	1,048 (148)	651 (149)
	240	5,216 (905)	1,857 (169)	61.4 (57.3 to 66.1)	1,131 (31)	724 (143)

a, Response to exercise; *b*, Control experiments (no exercise).
Figures are mean values for *a*, Six subjects; *b*, Four subjects. Standard deviation in parentheses except for % RFC where range is quoted.
WBC = white blood cells. RFC = rosette-forming cells.

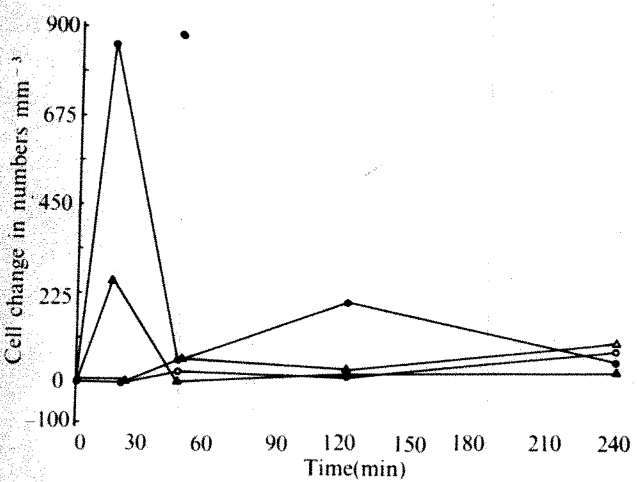

Fig. 1 Changes in total number of circulating RFC (T cells) (▲, △) and Non-RFC (B cells) (●, ○) following vigorous exercise (six experiments; ●, ▲) and in four controls (four experiments; ○, △). Pre-exercise level is taken as zero. Figures are calculated from electronic total white cell count, differential count of at least 500 stained leukocytes and mean of triplicate estimates for percentage of RFC.

ferent days from the same donors[17]. As the mean coefficient of variation for triplicate tubes in the rosette test was less than 5%, these findings seem to reflect a true and unpredictable fluctuation in the blood B:T cell ratio of healthy adults in conditions of normal daily activity.

In view of the demonstrated effect of strenuous exercise it seemed possible that the fluctuations observed in the control experiments were also related to physical activity. Samples were therefore obtained from five of the subjects in near-basal conditions (before rising after a night's sleep). These samples were handled as described above and the RFC:non-RFC ratio compared with the values obtained in random daytime samples obtained over a period of several weeks from the same donors (Table 2).

Table 2 Percentage of rosette-forming cells among blood lymphocytes of five healthy donors under 'basal' conditions and in random daytime samples

	Random samples*			'Basal' (early morning) samples		
Subject	Mean	(Range)	No. of samples	Mean (s.d.)†	or (range)	No. of samples
A	66.2	(61.8 to 69.4)	7	63.1 (2.3)		1
B	61.1	(53.5 to 67.7)	7	70.9 (3.3)		1
C	62.0	(51.9 to 70.3)	7	62.0	(55.7 to 72.5)	5
D	57.0	(53.8 to 61.0)	6	69.1 (2.2)		1
E	62.8	(54.4 to 68.4)	6	59.6 (1.6)		1

* Blood drawn at different times of day during normal activity, not involving strenuous exercise.
† Standard deviation of triplicate values for single blood sample.

In two cases (subjects B and D) the percentage of presumptive T cells in the early morning samples was greater than in any of their random specimens but in the other three subjects the figures obtained under near-basal conditions lay close to the mean values recorded during normal daily activity. When repeated early morning samples were examined from subject C, their variation in percentage of RFC was comparable to the variation observed in random daytime samples.

It therefore seems that the physical exertion undertaken in normal non-manual working conditions contributes little to the physiological fluctuations in the peripheral blood B:T cell ratio in health. The fluctuation does not follow any predictable time course and, apart from strenuous exercise, its causes have yet to be defined.

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Promotion of Reddening in Unripe Strawberry Fruits by Fungal Extracts

ETHYLENE promotes ripening in many fruits[1,2], and auxins cause premature colouring in pears[3], apples[4,5] and other fruits[6]. Some pome fruits redden prematurely around damaged areas.

No reports have been found of promoted ripening or reddening of strawberry fruits by ethylene, auxins or damage, and attempts elsewhere[7,8] and at Long Ashton, to advance ripening in strawberry by ethylene and by 2-chloroethylphosphonic acid (CEPA or Ethrel), which decomposes to yield ethylene in plant tissues, have not been successful, although tests have been done under several conditions, both *in vitro* and on fruit retained on the plant.

This investigation arises from the finding of several immature strawberry fruits, of the cultivar Rabunda, on which areas surrounding pathogenic infections were prematurely reddened. Lesions were pale-brown in colour, dry and firm, varying in size from about 1 to 3 cm^2 in area. The reddened areas surrounding the lesions formed a band up to about 1 cm in width. The remaining parts of the fruit were green and unripe at the time, but ripened later. A fungus was isolated from an infected fruit and identified as *Fusarium sambucinum* Fuckel, of importance on hops and stored potato but also recorded on strawberry in Europe[9].

Cultures of the fungus were incubated either on potato dextrose agar for 14 d, or in Czapek Dox liquid media for 15 d at 25° C. Six agar cultures in 9 cm Petri dishes were macerated in 100 ml methanol and the liquid phase separated. The methanol was evaporated off at 40° C under nitrogen. The water fraction was made up to 100 ml with distilled water and its *p*H adjusted to 2.5 with 1% HCl. This water extract was partitioned three times against 50 ml of ethyl acetate and the ethyl acetate phases dried with a little anhydrous sodium sulphate. The ethyl acetate was evaporated off at 40° C and the residue preserved for bioassay. Liquid cultures were extracted in a similar way.

The residues were assayed for their ability to colour unripe strawberry fruits *in situ* on plants in the glasshouse. Suitable fruits were firm but not hard, pale-green in colour, and 5 to 7 d before the stage at which they would normally colour.

Individual achenes were removed from the fruits with a sterilised scalpel, and a small cavity opened up beneath the scar. The residues to be assayed were taken up in 20% ethanol in water, and 8 µl aliquots placed into freshly-opened cavities. Treatments and controls (the latter included application of 20% ethanol and extracts of uninoculated agar and untreated cavities) were replicated from twice to ten times in different tests.

Active extracts applied in the manner described induced a ring of precocious reddening in tissue round the cavity 1 to 4 d after application, the response time depending partly on the time of year. The rings of reddened tissue were 2 mm wide and up to 1 cm in diameter, the diameter being less with greater dilution of the extracts. The cavity was separated from the ring by a zone of decoloured tissue, which did not ripen. Normal ripening of the fruit occurred outside the ring, which was no longer distinguishable, 3 to 5 d later. Control cavities exhibited neither red rings nor decoloured zones: the lip and lining inside the cavity turned brown but did not rot.

The dependence of ring diameter on extract concentration and the presence of the inner zone of decoloured tissue, indicate that the extracts may have been toxic in high concentrations near the point of application but became diluted by diffusion outwards to the region where they induced pigment formation in the host tissue.

Bioassay on *Avena* mesocotyls indicated that the extracts contained growth-regulating substances.

In work of colleagues in this laboratory, similar rings of reddened tissue were induced by the application of several auxins. Therefore, it appears likely that the fungal extract contained auxins or possibly other unidentified substances which can initiate or promote reddening or ripening in strawberry. The failure of attempts to promote ripening by ethylene or ethylene generators, however, raises the question whether ethylene was mediating in the stimulation of reddening either by auxins or the fungal extract, even though auxins are known to stimulate ethylene evolution in several plant tissues[10,11] and ethylene to promote ripening in many fruits.

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Negative Cooperativity of Phosphofructokinase as a Possible Regulator of Ripening in Banana Fruit

THE banana belongs to a group of fruits which undergo a rapid increase in respiration with ripening[1]. Several theories have been advanced to account for this 'respiratory climacteric'[2]: the most often mentioned hypotheses are (1) the uncoupling of oxidative phosphorylation, (2) a change in acceptor control by ADP, (3) a change in membrane permeability, and (4) the synthesis or activation of a rate-limiting enzyme. The uncoupler theory and the ADP to ATP ratio change have, however, been disproved, and no clear evidence has been presented in support of the other theories.

In lacatan bananas a twenty-fold increase in the fructose-1,6-diphosphate level has been measured and it has been suggested that this could indicate the activation of phosphofructokinase[3]. Activation of this same enzyme by an increasing concentration of inorganic phosphate has been suggested as a possible reason for increased respiratory activity in ripening tomato fruit[4]. Our study reports the regulatory control properties of phosphofructokinase during ripening of banana fruit.

The respiration of individual banana fruits *Musa cavendishii* Lambert var. Valery was followed with an infrared gas analyser in a continuous flow system. Fruit were removed from the respirometer (1) in the preclimacteric stage, (2), on respiratory rise, (3) at climacteric peak, and (4) at the two-day post-climacteric stage; they were then peeled and the pulp was grated into liquid nitrogen. Frozen tissue was lyophilised and stored at − 12° C. The dry pulp tissue was ground to a fine powder in a dry mortar. The powder for each of the four stages was extracted twice with extraction medium for the determination of endogenous metabolite levels. The extraction medium consisted of 50 mM tricine buffer, *p*H 8.5, 5 mM diethyldithiocarbamate, 5 mM dithiothreitol, 50 mM magnesium acetate and 0.2% Triton X-100. Stage 1 and stage 3 powders were extracted with the same medium and purified five-fold for phosphofructokinase assays.

Quantitative analysis of glycolytic intermediates showed the greatest relative change (twenty-fold) in the level of fructose-1,6-diphosphate between the preclimacteric stage to the climacteric peak; fructose-6-phosphate and glucose-6-phosphate, however, showed less than a one-fold increase (Table 1). This is consistent with phosphofructokinase activation hypothesis.

Table 1 Changes in levels of some glycolytic intermediates between preclimacteric and climacteric peak stage

Substrate	Preclimacteric (nmol g^{-1})	Climacteric peak (nmol g^{-1})	Change (%)
Glucose-6-phosphate	443 ± 23	792 ± 40	+79
Fructose-6-phosphate	444 ± 17	565 ± 30	+27
Fructose-1,6-diphosphate	7 ± 2	147 ± 11	+2,100
Triosephosphate	61 ± 16	227 ± 10	+372
Phosphoenolpyruvate	163 ± 9	533 ± 17	+327
Pyruvate	125 ± 8	366 ± 5	+293

Mean and standard error of duplicate samples are given.

The response of a partially purified phosphofructokinase from the preclimacteric (stage 1) and climacteric peak (stage 3) powder to increasing fructose-6-phosphate concentration, is shown in Fig. 1. The preclimacteric enzyme yielded a substrate saturation curve which is neither hyperbolic nor sigmoidal, whereas the climacteric peak enzyme displayed a more nearly hyperbolic pattern. The enzyme from both sources required very high substrate concentration to reach saturation. Figure 1 also shows that citrate, which has been shown to inhibit phosphofructokinase from carrot[5] and pea[6], did not inhibit at 5 mM concentration. A small inhibitory effect at very low fructose-6-phosphate concentration is shown in the insert to Fig. 1, but this is not large enough to regulate respiration. Other compounds reported as allosteric effectors of phosphofructokinase, such as inorganic phosphate, ammonium ion, ATP and ADP, showed no response or gave the same response with the enzyme from both sources. Plotting the data according to the Eadie equation[7] yielded curves which were concave upward (Fig. 2). The slow rate of substrate saturation and the shape of the Eadie plots are most consistent with the kinetic behaviour described as negative cooperativity[8]. According to this model the binding of one molecule of substrate makes the binding of the next molecule at another site on the same enzyme more difficult. This will maintain metabolism of carbohydrate through glycolysis at a low rate in spite of large changes in substrate. The pentose phosphate pathway, which provides NADPH and intermediates for RNA synthesis, can therefore proceed relatively independently of glycolysis.

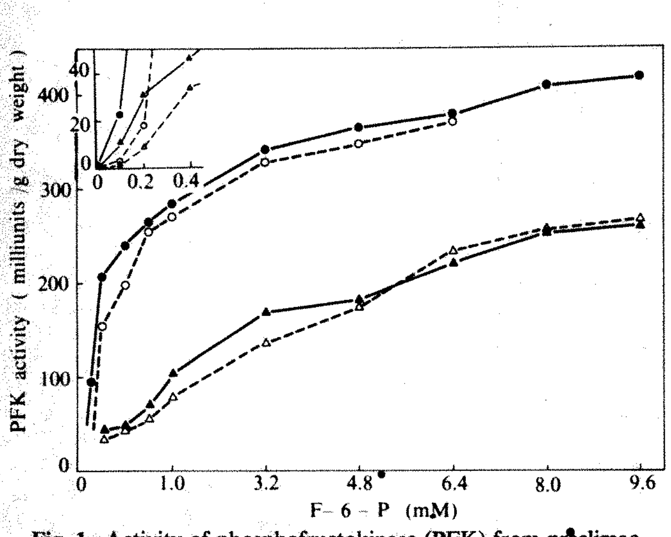

Fig. 1 Activity of phosphofructokinase (PFK) from preclimacteric and climacteric peak banana fruit as a function of fructose-6-phosphate (F-6-P) concentration. ●—●, Climacteric peak; ○—○, with 5 mM citrate; ▲—▲, zero time; △—△, with 5 mM citrate.

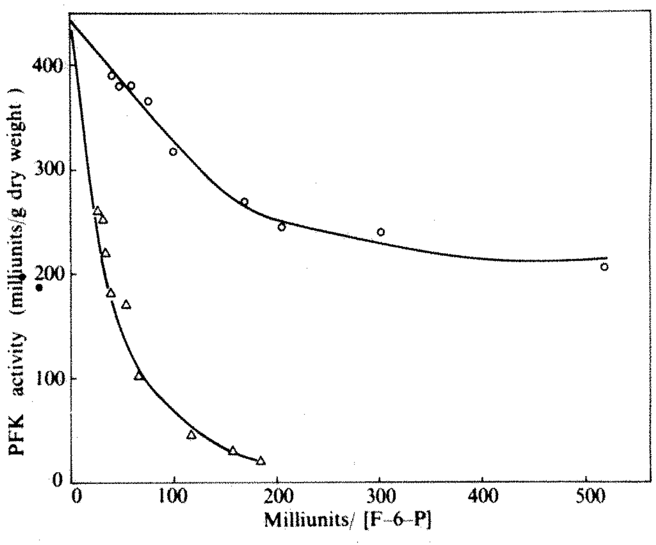

Fig. 2 A plot of the data of Fig. 1 according to the Eadie equation, $v = V_{max} - K_m (v/S)$.

The estimates of the V_{max}, the substrate concentration giving maximal reaction velocity, based on the Eadie plots, indicated that both the preclimacteric and the climacteric peak enzyme had the same value: 412 milliunits per g dry weight of powder. The $(S)_{0.5}$ value, the substrate concentration giving half-maximal activity, however, was 5 mM for the preclimacteric and 1 mM for the postclimacteric phosphofructokinase. We suggest that there is neither synthesis of a new kind of enzyme nor of the same enzyme, but rather that the activity of preclimacteric phosphofructokinase is kept low by the negative cooperative effect. This control is partially desensitised in some unknown way at the initiation of the climacteric.

Extraction procedures have been known to desensitise regulatory enzymes. We feel confident that control of the climacteric peak enzyme has not been affected by the procedures used because the enzyme does show some degree of control which is reproducible from experiment to experiment. The purification procedure includes an ammonium sulphate fractionation and a 3 min, 59° C heat treatment which leaves the control properties of both enzymes unchanged. Further, *in vivo* product accumulation agrees with the change in control of the extracted enzymes.

To our knowledge this is the first case where negative cooperativity has been shown in phosphofructokinase. Our results indicate that the behaviour of the banana phosphofructokinase is consistent with the activation hypothesis and that it can account for the observed five-fold increase in the respiration during the climacteric.

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Glycoprotein in the Capsid of Plant Viruses as a Possible Determinant of Seed Transmissibility

IT is well established that only certain plant viruses are transmitted by the seed, but little is known about how a virus invades the embryo and is subsequently transmitted to progeny[1,2]. Since viral coat proteins are determinants of host[3] and vector[4] specificity, we reasoned that they could also determine transmissibility. Accordingly, coat proteins of two seed-transmitted viruses, barley stripe mosaic virus (BSMV) and cowpea mosaic virus (CPMV), and three non-seed-transmitted viruses, tobacco mosaic virus (TMV), bromegrass mosaic virus (BMV) and bean pod mottle virus (BPMV)[1,2], were analysed.

BSMV, BMV and TMV were purified by centrifugation through multiple sucrose density gradient columns similar to those used in methods described previously[5]. CPMV and BPMV were purified by chloroform–butanol extraction, three cycles of differential centrifugation[6] and sucrose density gradient electrophoresis. Purified virus was not further separated into its electrophoretic forms[7]. Viral coat protein was isolated from highly purified BSMV and BMV by 1 M $CaCl_2$ (ref. 5), from CPMV and BPMV by the guanidinium LiCl method of Wu and Bruening[8] and from TMV by the acetic acid method of Fraenkel-Conrat[9]. The coat protein from each virus was analysed for carbohydrate as described in Table 1.

Table 1 shows the carbohydrate composition of the two seed-transmitted viruses, BSMV and CPMV. Both contained glucose, mannose, glucosamine and galactosamine, while xylose was present only in BSMV and galactose only in CPMV. Sialic acid, a common component of animal glycoproteins, was not detected. The coat proteins of the three non-seed-transmitted viruses contained no detectable carbohydrate.

Conditions for optimal carbohydrate release vary with each glycoprotein and depend on the stability of the glycoside linkages as well as the resistance to acid degradation of the released sugar residues. The hydrolysis conditions noted in Table 1 have been used successfully for various glycoproteins[10]. Even so, the values noted are minimum values since they represent the difference between the quantity of sugar released and quantity degraded during hydrolysis. Hydrolysis conditions normally used for amino acid analysis (6 N HCl, 100° C, 24 h) have been shown to degrade amino sugars extensively[10]. Our amino acid analysis of BSMV showed only a trace of amino sugars after 12 h of hydrolysis, and absence after 24 and 72 h of hydrolysis. This probably explains why previous amino acid analyses of BSMV[5] and CPMV[7] detected no amino sugars.

Table 1 Carbohydrate composition of BSMV and CPMV

	BSMV		CPMV	
Carbohydrate*	g per 100 g coat protein	Mol per virus particle	g per 100 g coat protein	Mol per virus particle
Glucose	1.36	1,830	0.51	136
Mannose	0.25	336	0.14	37
Xylose	0.38	614	—	—
Galactose	—	—	0.19	51
Glucosamine	0.66	891	0.70	187
Galactosamine	1.06	1,430	0.36	297
Sialic acid	0.0	0.0	0.0	0
Total	3.71	5,101	1.90	708

* Neutral sugars analysed by gas liquid chromatography of alditol acetate derivatives[19] after hydrolysis in 0.5 N H_2SO_4 containing Ag-50 × 8 (H^+), 100° C, 24 h; amino sugars were analysed by amino acid analyser and by gas-liquid chromatography of aminitol acetate derivatives[19] after hydrolysis in 4 N HCl, 100° C, 24 h; sialic acid was analysed using thiobarbituric acid[20] after hydrolysis in 0.1 N H_2SO_4, 80° C, 1 h. The alditol and aminitol acetates were chromatographed in a 2.6 mm 2 m glass column containing 3% ECNSS-M on 100/120 Gas-chrom Q isothermally at 180° C for the alditol acetates and at 200° C for the aminitol acetates. The quantity of each sugar was determined by comparing the area under its peak with that of the internal myo-inositol standard. The identity of each peak was confirmed using gas liquid chromatography–mass spectroscopy by comparing its retention time and computer print-out of the mass spectrum with known standards.

BSMV is a 128 × 20 nm rod[11] composed of 1,100 identical protein subunits[12] which account for 96% (w/w) of the virus particle[13]. The 3.71% carbohydrate in the coat protein indicates that this virus may contain up to 5,000 mol of carbohydrate per virion. CPMV in a 28 nm diameter icosahedral particle[14] composed of 180 protein subunits which account for 71.5% (w/w) of the virus particle[15]. Correspondingly, the 1.90% carbohydrate in the coat protein suggests that this virion contains up to 700 mol of carbohydrate. The presence of these rather large amounts of carbohydrate could convey to these viruses a unique capacity for interaction with their hosts.

It is significant that the two viruses with a glycoprotein coat are seed-transmitted while the three viruses devoid of carbohydrate residues are not[1,2]. Furthermore, CPMV and BPMV are very closely related in that they: (*a*) cross-react serologically[16]; (*b*) have similar host ranges; (*c*) have a similar complement of fast and slow electrophoretic forms and subunit proteins[10,17], and (*d*) have similar amino acid compositions in their coat proteins[10,17]. Thus when the properties of these two closely related viruses are compared it seems particularly relevant that seed-transmitted CPMV has a glycoprotein coat, while non-seed-transmitted BPMV has a coat protein devoid of carbohydrate.

Because viral invasion of gametophytic tissues is a prerequisite for seed transmission[1,2,18], seed-borne viruses seem to possess a unique property which enables them to invade reproductive tissues. We suggest that this unique property of seed-borne viruses is related to the glycoprotein portion of the coat protein. More specifically, the covalently linked carbohydrate residues on the viral coat may function as recognition sites to attach the virus to gametophytic cell surfaces and thereby facilitate viral invasion of reproductive tissues and subsequent seed transmission.

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Glutathione Peroxidase Activity as a Function of Dietary Selenomethionine

Since 1957, selenium has been recognized as an essential micronutrient for animals, but its exact biological function has remained uncertain. The metabolic role of selenium in animals seems to be linked with vitamin E and sulphur amino acids[1-4]. Selenium has a sparing effect on vitamin E, and delays the onset of deficiency syndromes. Likewise, vitamin E and sulphur amino acids partially protect against or delay the onset of several forms of selenium deficiency syndromes. Rotruck *et al.*[5] proposed that selenium functions as an integral part of glutathione (GSH) peroxidase, an enzyme that reduces toxic lipid peroxides to hydroxy acids[6-8]. They found that a large proportion of the ^{75}Se from rat erythrocytes labelled *in vivo* remained with the enzyme during extensive purification, and more recently, their studies with highly purified GSH peroxidase have indicated that it contains approximately 4 mol of Se per mol of enzyme[9]. Similar findings were reported by Flohé *et al.* for bovine erythrocyte GSH peroxidase[10]. To learn more about the nutritional relationship between selenium and GSH peroxidase, we performed studies that showed GSH peroxidase activity is proportional to dietary selenium, which provided additional evidence for the role of selenium in the function of GSH peroxidase.

Weanling male Sprague-Dawley rats were fed a selenium-deficient diet that contained sucrose, 43%; *Torula* yeast, 36%; stripped corn oil, 10%; stripped lard, 5%; salt mix, 5%; and vitamin mix, 1%. This diet was supplemented with 0, 0.05, 0.2, or 2.0 p.p.m. selenium as selenomethionine. After 5 weeks, the animals were exsanguinated by heart puncture. The heparinised blood was centrifuged at 3,000 r.p.m. for 25 min. The packed cells were washed with isotonic buffer and lysed with hypotonic phosphate buffer. The tissues were stored frozen pending analysis. Tissues from individual animals were prepared as approximately 6% homogenates in isotonic potassium phosphate buffer, *p*H 7.0 After centrifugation for 10 min at 750*g*, the supernatant was recentrifuged for 60 min at 100,000*g* to obtain the soluble fraction. GSH peroxidase activity in the soluble fraction was assayed at *p*H 7.6 by a modification of the method of Paglia and Valentine[11], with cumene hydroperoxide as substrate. The activity was measured spectrophotometrically as the rate of NADPH oxidation through the coupled reaction with excess GSH reductase. Previous work showed that the activities of GSH reductase and glucose-6-phosphate dehydrogenase, as well as GSH peroxidase, increased significantly in response to oxidative stress[12,13]; therefore, these enzymes were measured as controls[14,15] to show the specificity of selenium for GSH peroxidase. Protein concentration in the soluble fractions was also determined[16].

In all tissues, dietary supplementation with 0.2 p.p.m. and 2 p.p.m. selenium significantly ($P<0.01$) increased the activity of GSH peroxidase over the activity in tissues from non-supplemented controls. Additionally, supplementation with 0.05 p.p.m. selenium significantly ($P<0.01$) increased the enzyme activity above that of non-supplemented animals in all tissues except liver. Linear regression analysis showed that the increased GSH peroxidase activity was a function of the logarithm of the dietary selenium concentration (Fig. 1). All correlation coefficients were above 0.7 and all $P<0.001$. The relative increase of GSH peroxidase activity with dietary selenium supplementation during the experimental period was greatest in plasma, with other tissues following in the order liver>heart>kidney>erythrocytes≃lung>muscle. The close relationship between dietary selenium and tissue GSH peroxidase activities, particularly in the plasma, could be used as the basis for a rapid monitoring method for the selenium status of an animal. In all tissues studied, the activities of GSH reductase and glucose-6-phosphate dehydrogenase were unaffected by dietary selenium.

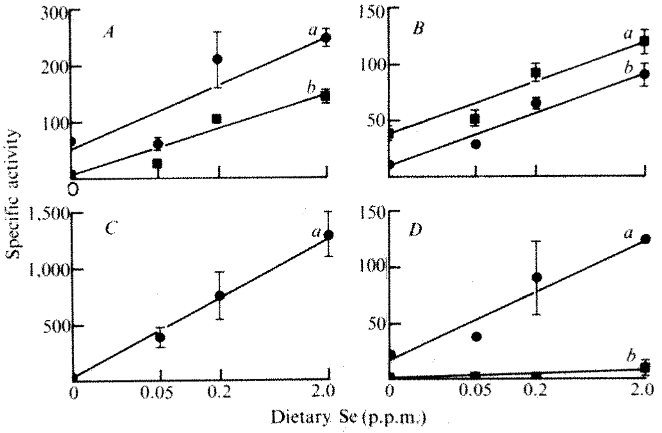

Fig. 1 Relationship of GSH peroxidase activity to dietary selenium. Specific activity is expressed as nmol NADPH oxidised per min per mg of protein for all tissues. Plasma-specific activity is expressed as nmol NADPH oxidised per min per ml. Each data point is the mean for nine animals in the 0 p.p.m. selenium group and for five animals in each selenium supplemented group. Bars represent ± standard deviation, points that lack bars have standard deviations smaller than the graphical representation of the data point. *A*: *a*, Liver, *b*, heart. *B*: *a*, RBC; *b*, lung. *C*: *a*, Plasma. *D*: *a*, Kidney; *b*, muscle.

A similar specific effect of dietary selenium on GSH peroxidase activity was observed in chicks[17] (S. T. Omaye, unpublished results). The mechanism of enhancement of GSH peroxidase activity has not been elucidated, although it is suspected to occur through increased synthesis of GSH peroxidase. In support of this mechanism for the enhancement of activity, our experiments indicate that selenium is not a dialysable or exchangeable cofactor; it is reported to be an integral part[9,10] of the enzyme.

Use of similar nutritional studies to yield evidence of the involvement of a trace element with a specific enzyme is well documented[18]; for example, xanthine oxidase in rats fed a highly purified diet was shown to be proportional to dietary supplementation with a liver residue factor that later was identified as molybdenum[19].

The critical dependency of GSH peroxidase activity on dietary selenium may explain the long observed and puzzling interactions among dietary selenium, vitamin E and sulphur amino acids. GSH peroxidase, especially in the absence of dietary vitamin E, plays a major role in detoxifying lipid peroxides[6–8,12] by reducing them to non-toxic hydroxy fatty acids, which prevents their decomposition into free radicals that can reinitiate peroxidation. The antioxygenic action of sulphur amino acids[3,20] can be explained in part by their involvement in the production of GSH, which is linked through GSH reductase to the NADPH generating system of the cell. Hence, dietary selenium, sulphur amino acids, and vitamin E act synergistically to protect tissues from oxidative damage.

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Zooplankton Feeding on Differentially Labelled Algae and Bacteria

HERBIVOROUS zooplankton can graze on planktonic algae, bacteria and detrital particles[1]. Selective feeding of planktonic crustaceans on algae has been described and attributed to passive size selection by filtration or raptorial feeding[1]. The ingestion and utilisation of algae, bacteria and detritus by zooplankton has also been noted[2–6] but as yet there have been few reports concerning the behaviour of zooplankton grazing on mixtures of algae and bacteria.

The potential importance for herbivorous zooplankton of food sources other than phytoplankton was demonstrated by Provasoli, Shiraishi and Lance[7] who found that axenic cultures of the shore pool Copepod, *Tigriopus*, which did not remain viable for many generations if grown on a single strain of bacterial free algae, could be maintained indefinitely when their diet was supplemented with bacteria. In natural waters both the quantity and quality of zooplankton food sources are critical factors which are difficult to evaluate by present techniques[8]. Here we describe a method of differential radioactive isotopic labelling of algae and bacteria for studies of zooplankton grazing patterns and present some preliminary results using this method with zooplankton from Lake Kinneret (The Sea of Galilee), Israel.

During the summer, in the metalimnion of Lake Kinneret, the photosynthetic, green sulphur bacterium, *Chlorobium phaeobacteroides* can reach concentrations as high as 10^7 cells ml^{-1}. Large numbers of Cladocerans and early stages of Copepods are generally found in the upper metalimnic layer at this time[9]. Feeding experiments showed that the nauplii stages of the most abundant Copepod (*Mesocyclops leukartii*, Claus) developed normally when fed on *Chlorobium* isolated in pure culture on the enrichment medium described by Pfennig[10]. The rate of nauplii development on a *Chlorobium* diet was more rapid than on other bacterial and algal food sources (Table 1). It seemed reasonable, therefore, to investigate the possibility that, in this lake, as elsewhere[11–13], the large population of photosynthetic bacteria was being utilised as a food source by the zooplankton. Subsequent feeding experiments were made with *Ceriodaphnia reticulata* (Jurine) because this Cladoceran is one of the most common herbivorous zooplankters in Lake Kinneret. *Ceriodaphnia* and *Daphnia magna*, when taken from the lake, survived and reproduced normally for at least several weeks on diets of *Chlorobium*, *Chlorella vulgaris* or *Chlamydomonas reinhardii* in filtered Kinneret water.

Table 1 Development time (d) in nauplii of *Mesocyclops leukartii* (Claus) on various food sources*

Food	Incubation temperature 15° C	27° C
Chlorella vulgaris	37 (±4)	12 (±2)
Rice bacteria †	36 (±7)	9 (±2)
Yeast		11 (±1)
Chlorobium phaeobacteroides	26 (±10)	8 (±1)

*In each experiment, which was repeated at least twice, ten nauplii were incubated in 15 ml filtered lake water. Food sources were added in excess (such as *Chlorobium* 2.5×10^7 cells ml^{-1}) as determined experimentally. The development times were averaged for all organisms in each experimental situation. Standard deviations in parentheses.

† Bacteria obtained from rice infusion.

Chlorobium phaeobacteroides is capable of assimilating organic substrates such as glucose, amino acids or acetic acid in the light[14]. Therefore, for our experiments, we labelled the bacteria by growing them with ^{3}H-acetate (0.2 μC ml^{-1}) on Pfennig's medium[10]. Algal cultures (*Chlorella* or *Chlamy-*

Table 2 *Ceriodaphnia reticulata* feeding experiments with radioactively labelled algae and bacteria*

Experimental time (h)	No. of *Ceriodaphnia*	Food source (cells ml^{-1})		Assimilated cells per individual *Ceriodaphnia*‡	
		Algae	Bacteria	Algae	Bacteria
4	50	61.4×10^4	—	2.2×10^3 (0.18)	—
4	50	—	2.0×10^7	—	1.3×10^4 (0.033)
4	50	61.4×10^4	2.0×10^7	0.2×10^3 (0.02)	1.5×10^4 (0.037)
4	50	267.2×10^4	—	0.6×10^3 (0.11)	—
4	50	—	23.3×10^7	—	3.1×10^4 (0.007)
4	50	267.2×10^4	23.3×10^7	0.2×10^3 (0.037)	2.1×10^4 (0.005)
18	20	104.4×10^4	—	31.2×10^3 (0.60)	—
18	20	—	52.5×10^7	—	85.0×10^4 (0.32)
18	20	104.4×10^4	52.5×10^7	4.2×10^3 (0.08)	118.0×10^4 (0.45)
40	20	40.0×10^4†	—	15.0×10^4 (7.5)	—
40	20	—	0.6×10^6	—	0.6×10^4 (0.20)
40	20	40.0×10^4†	0.6×10^6	0.4×10^4 (0.2)	0.8×10^4 (0.26)
43	50	2.0×10^4	—	15.3×10^3 (38.2)	—
43	50	—	35.0×10^7	—	8.0×10^4 (0.11)
43	50	2.0×10^4	35.0×10^7	0.3×10^3 (0.75)	7.2×10^4 (0.10)

* Algae and bacteria were labelled with ^{14}C and ^3H respectively as described in the text. Algal cells were counted in a haemocytometer and bacteria by specific absorbance of bacterial chlorophyll at 715 nm or by serial dilutions in agar shake tubes. Radioactivity determinations were made with a scintillation counter using Instagel fluor (Packard). Initial activities ranged from 2 to 350 c.p.m. per 10^3 algal cells, and from 100 to 6,000 c.p.m. per 10^3 bacteria.

† *Chlamydomonas* (*Chlorella* were used in all other experiments).

‡ Percentage of food source utilised in parentheses.

domonas) were incubated with ^{14}C bicarbonate (1 μC ml^{-1}) added to a mineral growth medium (Bristol).

The results of five experiments with *Ceriodaphnia* feeding on algae, or bacteria, or both, are shown in Table 2. In each experiment twenty to fifty *Ceriodaphnia* were incubated with slow rotary agitation in 100 ml of filtered lake water, to which were added algae, or bacteria, or both. For each experimental situation, the radioactivity in three replicates of five animals was determined. The uptake of algae or bacteria is given as cells per individual *Ceriodaphnia*. These values might also be expressed in terms of fresh weight biomass (for example, 650 bacterial cells = one algal cell), carbon biomass, or calorific value. None of these parameters, however, reflect the actual dietary value of the algae and bacteria for *Ceriodaphnia*. No significant radioactivity was retained in control samples with animals poisoned by 4% formaldehyde.

In all experiments, when mixed diets were given, the *Ceriodaphnia* assimilated much less algae than when fed on algae alone. In contrast, the utilisation of bacteria was relatively unchanged when algae were also included in the diet. These experiments indicate, therefore, a preferential assimilation of bacteria by *Ceriodaphnia*. In all cases, both algae and bacteria were in excess supply, as indicated by the very low values for percentage utilisation of each food source.

In *Ceriodaphnia* the ingestion of food is a rapid process, taking less than 5 min (ref. 15). Because all the present experiments lasted 4 h or more, it is probable that the results shown in Table 2 actually represent assimilated foodstuff. Discriminatory grazing in *Ceriodaphnia* seems unlikely[1,15] and it would thus appear that the animals could more easily digest cells of bacteria than those of algae. One reason for this may be the relative ease with which the *Chlorobium* cells are lysed (Pfennig, personal communication) as contrasted to algae with cell walls of cellulose.

The above experiments give a preliminary indication of the preferential assimilation of bacteria by *Ceriodaphnia* and imply that the photosynthetic green sulphur bacteria of Lake Kinneret may indeed be a significant dietary source for zooplankton. More importantly, we illustrate that the simple technique of labelling algae with ^{14}C, and bacteria (or other heterotrophs) with ^3H can make possible more extensive and complex experiments to elucidate the patterns of zooplankton feeding in nature.

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Feeding Posture of Modern Stalked Crinoids

Most modern crinoids (Echinodermata) are comatulids, which lack the stalk characteristic of Palaeozoic crinoids. The specialisation and adaptation to different ecological niches made possible by loss of the stalk have resulted in several different ways of spreading the arms and pinnules in an array for suspension feeding. Until the 1960s, most observations were based on specimens in aquaria. These suggested that the comatulids sit on the substratum with the mouth orientated upwards, the arms stretched out in the form of a broad funnel or collecting bowl. The crinoid was thought to wait passively for a rain of its food material from above[1]. This feeding posture is used by some deep water crinoids[2,3]. Direct observations of the feeding behaviour of a shallow water comatulid in the Red Sea during the 1960s demonstrated that the arms are withdrawn during the day, but at night are arrayed in a vertical plane to form a filtration fan. This fan is orientated perpendicular to a prevailing weak current, filtering some 40,000 l of water at a current speed of 2 cm s^{-1} during one period of activity[4]. These observations and a review of deep-sea photographs indicated that modern crinoids favour an environment with moderate currents and are to some degree current-seeking (rheophilic)[5]. Application of these ideas to the palaeocology of Palaeozoic stalked crinoids suggested that most were rheophilic, using the stalk to raise the calyx above the substratum and allowing the arms to be outspread in a planar, circular filtration fan[2]. The morphology of some ancient forms, however, was considered indicative of a rheophobic mode of life.

Direct observations of the shallow water crinoids of the West Indies using Scuba has expanded and refined knowledge of the various comatulid postures[6]. Some rheophilic comatulids which form a filtration fan are attached to elevated objects and favour habitats swept by moderate currents. In these species, as in the Red Sea comatulid, the arms are held such that the food groove faces downcurrent. Other West Indian comatulids attach the calyx within a cryptic niche but exend the arms in a fan normal to bidirectional wave surge at shallow depths. Rheophobic species may co-exist with the rheophiles, attaching within the reef infrastructure and extending the arms in several directions. The pinnules of each arm are arrayed in four directions, thus allowing effective filtration of slow, multidirectional water movements[7]. The formation of collecting bowls dependent on gravitational settling is apparently restricted to deep water species[2,3].

In August 1972, an extended series of dives in the submersible Nekton Gamma on the north coast of Jamaica at Discovery Bay, revealed an abundant fauna of living stalked crinoids and comatulids at depths of 200–300 m, the latter being the maximum operating depth of the submersible.

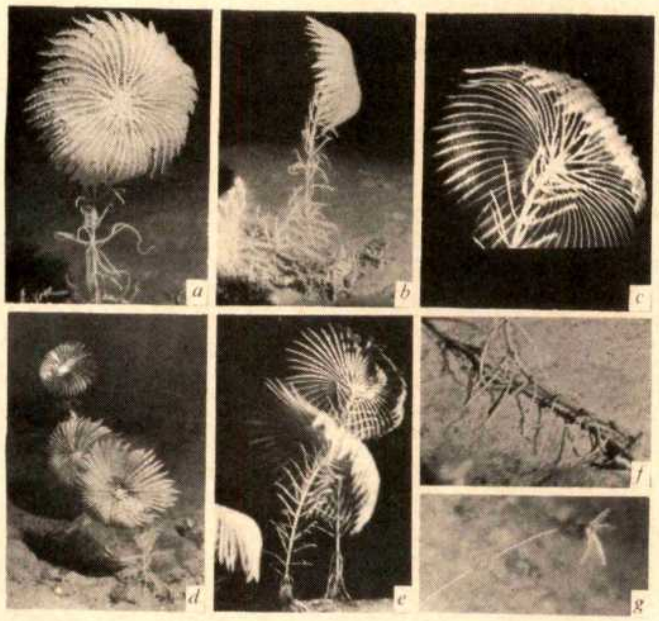

Fig. 1 *In situ* views of *Cenocrinus asterius* (L.) between 200 and 300 m depth, off Discovery Bay, Jamaica. Total vertical, height of animals approximately 1 m (*a–e*); *f*, basal part of stalk with cirri, supporting animal above substrate; *g*, *in situ*, view of *Democrinus* sp. between 200 and 300 m depth, off Discovery Bay, Jamaica. Total vertical height of animal 0.33 m.

Extensive direct observation and photography by ourselves and others has demonstrated the formation of filtration fans by three species. The most prominent and largest of these is *Cenocrinus asterius* (L.), with nearly fifty arms (Fig. 1*a*, *c*). The stalk may approach 1 m in length. The arms were always seen to recurve aborally through almost 270°. In this posture the repeated branching of the arms and the pinnules form a virtually continuous parabolic filtration fan. The calyx with arms recurved in this manner was observed with the mouth orientated laterally (Fig. 1*b*) or upwards (Fig. 1*e*), appearing in the latter case like a wilted flower. The lateral tilting of the calyx (Fig. 1*d*) suggests current moving from left to right, but direct observations indicate that in fact the arms are recurved into the current, even in currents exceeding 0.5 knot. Thus the food grooves of the arms are directed downcurrent as in some rheophilic comatulids. The 'holding' power of the ligaments in the stalk is sufficient to keep the calyx erect even in currents, as suggested by histological studies[6]. In conditions of slack current, the calyx is horizontal, with the mouth upward. Comatulid crinoids were observed attached to the stalk of the stalked species, also forming a filtration fan (planar rather than parabolic) orientated like that of the 'host' stalked crinoid. Other comatulids attached to the substratum were also observed forming filtration fans in the same area.

Three other stalked crinoids were also present. *Endoxocrinus parrae* (Gervais), another large multi-armed species, arrayed the arms in a parabolic filtration fan like that of *C. asterius*. The small, five-armed *Democrinus* sp. was abundant at 240–300 m. It also forms the fan, but it is not as dense a screen as that of the former species. The calyx was observed to bend downcurrent, with the fan extended against the flow (Fig. 1*g*). In contrast to the three long-stalked crinoids, *Holopus rangii* d'Orbigny did not form a filtration fan. This species occurs commonly at depths of 270–300 m off Discovery Bay, cemented like barnacles to vertical rock outcrops. The ten short, massive arms were observed extended in a funnel-like arrangement which was rapidly infolded on approach of the submersible, giving the appearance of a clenched fist.

Anchorage of the two large species, *C. asterius* and *E. parrae*, is achieved solely by the cirri along the stalk. The stalk curves onto the substratum and four or five groups of cirri grasp the substratum with the terminal claws (Fig. 1*f*). The stalk terminates abruptly and seems not to give rise to an actual root. Some cirri along the vertical part of the stalk near the substratum seem to act as props (Fig. 1*f*).

Photographs obtained (by C. Neumann, personal communication) from the submersible Alvin in the Straits of Florida at 550 m demonstrate that stalked crinoids form filtration fans with the food grooves downcurrent in that location also. The genera present seem to be *Endoxocrinus* and *Democrinus*. Some comatulids present also form filtration fans. Sediment shadows behind sponges and sponge growth forms confirm current directions.

Several conclusions are suggested by the observations in Jamaica and the Straits of Florida. The stalked crinoids of the West Indies usually form filtration fans for suspension feeding in areas swept by moderate currents. The presence of these species in deep-sea photographs and dredge samples suggest the occurrence of moderate bottom currents. The orientation of the arms establishes current direction. The cirri are the means of anchorage in the larger species, serving also as props for the stalk. The stalk ligaments provide suffisient rigidity to hold the calyx erect even under moderate currents. It is thus highly probable that Breimer's inferences[2] as to the rheophilic habits of many Palaeozoic crinoids are correct. Other specialised Palaeozoic stalked crinoids may have used some of the same diverse patterns as those of modern unstalked forms.

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Archaeocyathid-bearing Erratics from Dwyka Subgroup (Permo-Carboniferous) of South Africa, and their Importance to Continental Drift

THE Dwyka Subgroup, forming the basal division of the Karroo Group, comprises a thick sequence of glacial morainic debris and boulder shales, together with subordinate shales, varvites, and lenticular sandstones. In the southern Cape, tillitic rocks are overlain by the so-called "Upper Dwyka shales", an argillaceous sequence at the top of which are white-weathering carbonaceous shales, the "White Band". The latter is overlain by a thin but persistent chert band which forms the arbitrary boundary with the overlying Ecca Subgroup. The Upper Dwyka shales have yielded all the animal fossils of this subgroup in the southern Cape. McLachlan and Anderson[1] have recorded orthocerid nautiloids, the brachiopod *Attenuatella*, the bivalves *Phestia* and (?)*Nuculopsis*, palaeoniscoid fish, radiolarians, spiral coprolites suggestive of the presence of sharks, fossil wood, foraminifers, and miospores from the base of the succession near Kimberley. They[2] favoured a Sakmarian age for this marine incursion. The non-marine[2] White Band has yielded the aquatic reptile *Mesosaurus* and the crustaceans *Notocaris*, *"Pygaspis"*, and *Anthrapalaemon*.

Glaciomarine beds spanning some 250 m at the base of the Dwyka succession in South West Africa[3-5] have yielded the ammonite *Eoasianites* (*Glaphyrites*), an orthoconic nautiloid, possibly *Dolorthoceras*, the bivalves *"Aphanaia"* and *Eurydesma*, the gasteropod *Peruvispira*, the coelenterate *Conularia*, the echinoid *Archaeocidaris*, the ectoproct *Dyscritella*, the palaeoniscoid fish *Namaichthys* and *Acrolepis*, a monasterid starfish, as well as crinoid stems, an indeterminate brachiopod, foraminifers, radiolarians, and fossil wood. *Mesosaurus*, as in the southern Cape, occurs only in white-weathering shales at the top of Dwyka succession[3,6].

In view of the relative scarcity of fossils within the strata of this Subgroup, it is of great interest to record the discovery of fossiliferous glacial erratics from the Prince Albert district. This find by one of us (R. O.) is all the more significant for the fact that the fossils are Cambrian archaeocyathids (Fig. 1). They occur in grey limestone cobbles, representing transported glacial erratics, sporadically embedded in the tillites of the Dwyka Subgroup.

Archaeocyathids have a cosmopolitan distribution in Lower and Middle Cambrian marine deposits, during which period they built extensive reefs in Australia, North America, Europe, North Africa, Asia, and Antarctica. Nowhere are they known from younger deposits and the phylum is a short-lived one.

No marine rocks of Cambrian age are known from southern Africa. In South West Africa the Nama Group is of terminal Precambrian age[7], characterised by an "Ediacaran" faunal element. Among the fossil genera recorded are *Rangea, Nasepia, Orthogonium, Pteridinium, Cyclomedusa, Ernietta*, and so on. Unconformably overlying the Nama Group are the "red-bed" deposits of the Fish River Formation, formerly included within the Nama Group[8], but now considered to represent molasse deposition following the Damaran orogeny[7]. Martin[8] considers the most reliable age for the post-tectonic pegmatites intrusive into the Damara Group to be 510±60 Myr. It seems probable, therefore, that the Fish River Formation spans at least part of the Cambrian period, a suggestion supported by Germs's[7] record of *Phycodes pedum* Seilacher from these sediments. No undoubted marine fossils are known from these predominantly terrestrial red-beds, and they thus provide an important palaeoclimatic marker for the Cambrian period in southern Africa.

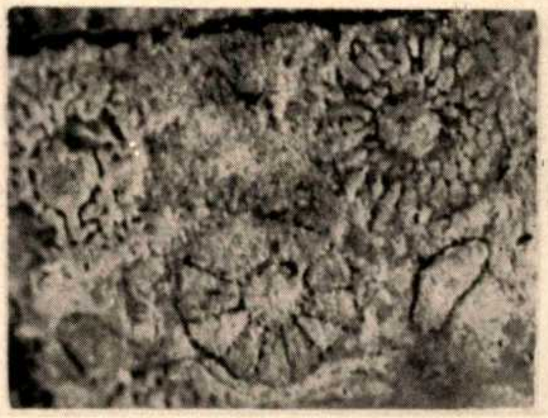

Fig. 1 Archaeocyathids from limestone cobbles embedded in the Dwyka tillite, approximately ×5.

In the north-western Cape, at Vanrhynsdorp, the Table Mountain Subgroup rests unconformably upon Nama strata, while in the southern Cape it is conformably succeeded by the marine strata of the Bokkeveld Subgroup, dated at lower Devonian (early Emsian)[9]. The recent assignment of an Upper Ordovician age (Upper Ashgill)[10] to a brachiopod assemblage from the Cedarberg Formation suggests that the orthoquartzites of the Nardouw Formation themselves span the entire Silurian period, as well as the base of the Devonian (Fig. 2). An Upper Cambrian age for the basal beds of the Table Mountain Subgroup thus seems likely, and is supported by the red-bed deposits of the basal Graafwater Formation, in the absence of the conglomerates of the impersistent Piekenier Formation.

The Table Mountain Subgroup is separated by a regional disconformity from the underlying Klipheuwel Formation[11]

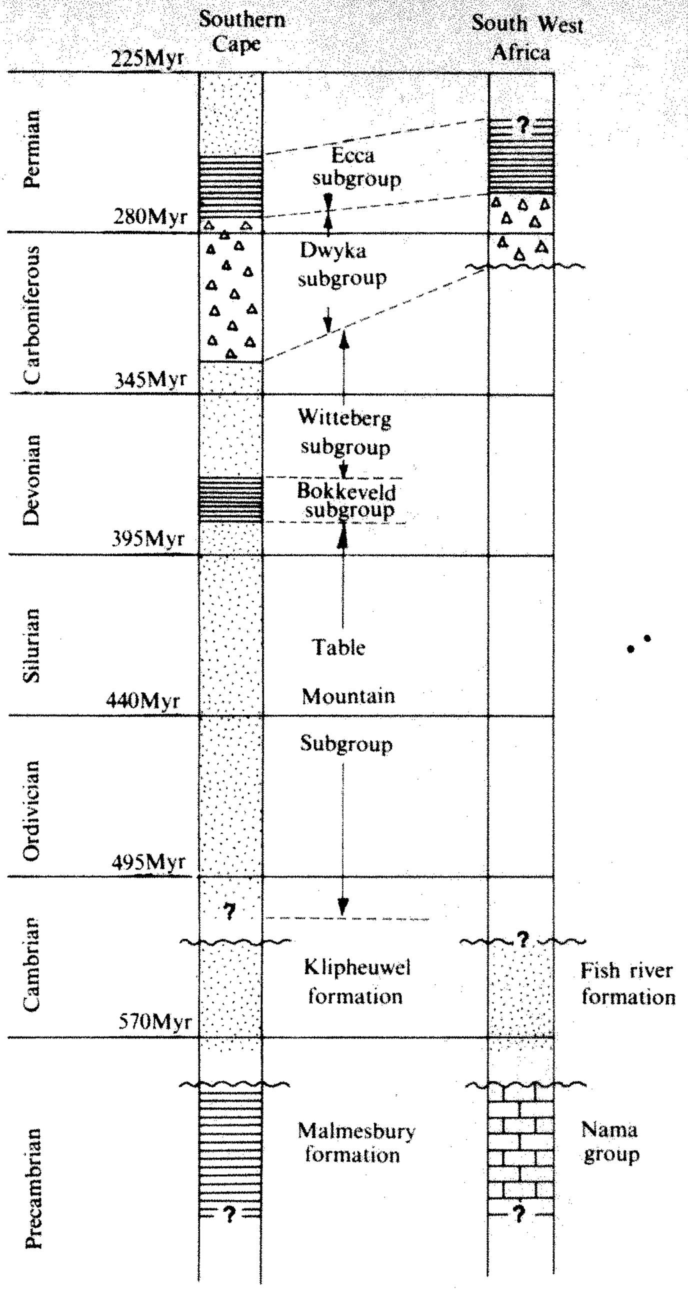

Fig. 2 The Palaeozoic chronostratigraphy of southern Africa.

in the southern Cape, the latter comprising brightly coloured greywackes and shales, and representing typical red-bed sediments. These sediments represent the lithostratigraphic, and in all probability, the chronostratigraphic equivalents of the Fish River Formation. Unfortunately they have yielded no fossils.

A study of the Cambrian deposits of southern Africa shows deposition to have been affected under terrestrial red-bed conditions, and thus most certainly not a source for wholly marine archaeocyathids. Where did these fossils come from? Their occurrence in glacial erratics suggests transport from a distant source area. It is necessary, therefore, to consider the palaeo-ice-flow directions of the Dwyka tillite.

Stratten[12], from a study of till fabric and glacial striae, considered the glacial deposits of the Prince Albert area (Fig. 3) to have been derived from the west (the Atlantic Ice Sheet), the north (the Namaland and Transvaal Ice Sheets), and the south (the Southern Cape Ice Sheet). Of these, the northerly sources can be excluded since, as just shown, no marine Cambrian is known from southern Africa. Recent evidence[13] suggests that the westerly source, that is, the Atlantic Ice Sheet, is incorrect, and that the glacial ice-flow direction was actually towards the west, thereby corresponding with known palaeo-ice-flow directions in South America. This leaves only the Southern Cape Ice Sheet as the transporting agent. On the Gondwana reconstruction (Fig. 4) this ice flow had its origin in Antarctica. It is of extreme interest, therefore, not only to note the occurrence of *in situ* archaeocyathid limestones in the southern Argentina Range and from the Nimrod Glacier—Beaumont Bay region of southern Victoria Land[16], but also their occurrence in moraine boulders at Mount Spann[17].

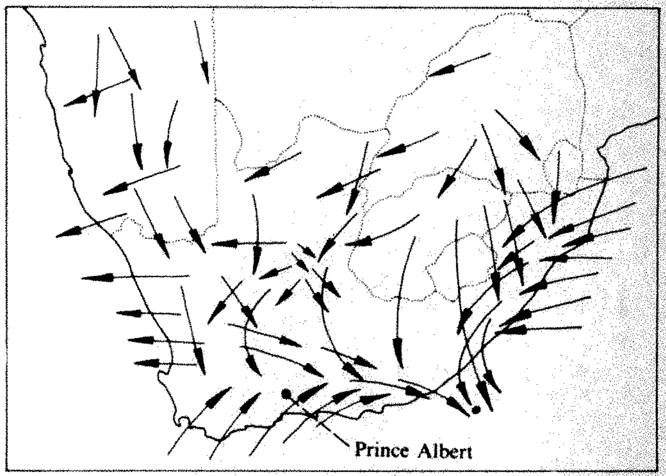

Fig. 3 Dwyka ice-flow directions. After Stratten[12] and Theron and Blignault[13].

Thus the only feasible source for the archaeocyathids embedded within the tillites of the Dwyka Subgroup seems to be Antarctica, thereby providing unequivocable evidence for the former unity of the southern continents.

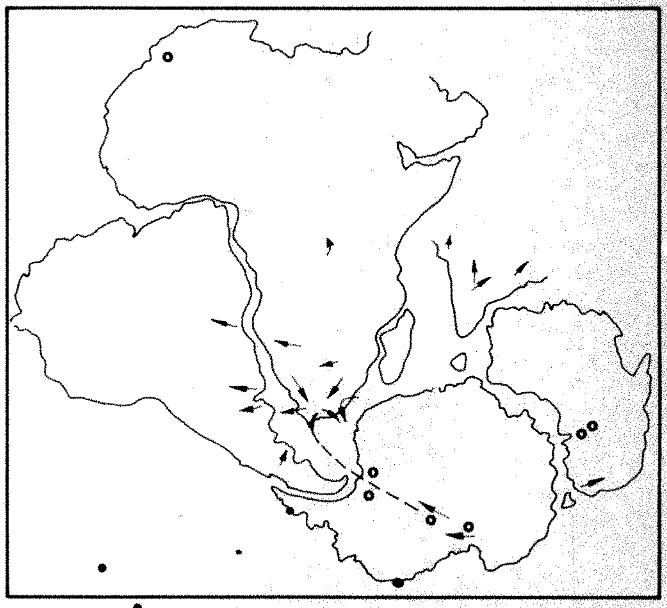

Fig. 4 The Gondwana distribution of archaeocyathids (circles), together with the late Palaeozoic ice-flow directions. After Stratten[12], Theron and Blignault[13], Ahmad[14], and Lindsay[15].

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Discrimination learning in ascorbic acid-deficient guinea pigs

PAULING[1] has suggested that a high intake of vitamin C may be necessary for optimum cerebral function including learning and memory. We have tested this hypothesis in a preliminary way by comparing maze learning in two groups of guinea pigs: those receiving large supplements of ascorbic acid (the 'control' group) and those receiving a daily quantity of the vitamin sufficient to maintain a concentration in the brain at approximately 25% that of the controls (the 'deficient' group).

Thirteen male guinea pigs were reared on a diet well supplemented with ascorbic acid. When the animals were 9 weeks old (day 0) they were randomly assigned to one of two groups ('control' or 'deficient') and given an ascorbic acid-free diet[2]. Drinking water was changed daily. That of the 'controls' contained 1% ascorbic acid throughout the experiment. From day 16, both groups received a daily oral dose of ascorbic acid (6 mg kg^{-1} body weight) just sufficient to maintain good growth in deficient animals[3,4]. Water consumption measurements indicated that controls received at least 500 mg kg^{-1} body weight of ascorbic acid daily. This intake is much in excess of that required to saturate guinea pig tissues with vitamin C[3].

From day 46 to day 67 animals were tested for spatial discrimination in a water T-maze (J. L. Smart, in preparation). On the first two days (days 46 and 47) escape was possible from both arms and animals were given five trials a day to test for left or right preferences. There followed 20 successive days of six trials per day during which escape was possible from only one arm off the maze. Animals were required to reverse previous preferred or learned responses. A learning criterion (successful 'reversal') was said to have been met when no errors (entries into arms other than the escape arm) were recorded during the last five of the six trials. The day after meeting this criterion the escape route was reversed.

TABLE 1 Body weight, tissue ascorbic acid and maze performance in control (saturated) and chronically ascorbic acid-deficient guinea pigs

		Control ($n = 6$)	Deficient ($n = 7$)
Body weight (g)	day 0	413 ± 61	420 ± 63†
	day 68	651 ± 40	626 ± 62†
Tissue ascorbic acid on day 68 (μmol g^{-1} wet wt)	Forebrain	1.11 ± 0.05	0.30 ± 0.05*
	Brainstem	0.70 ± 0.05	0.20 ± 0.04*
	Liver	1.67 ± 0.20	0.28 ± 0.17*
	Adrenals	6.49 ± 1.28	0.91 ± 0.15*
T-Maze performance (days 48 to 67)	Total reversals	7.0 ± 1.1	6.1 ± 2.9†
	Total errors	50.0 ± 11.0	48.7 ± 13.4†

Results are mean ± s.d.
* $P < 0.001$.
† Not significant (Student's t test) when compared with corresponding control value.

On day 68 animals were killed by decapitation and ascorbic acid was determined[5] in brain, liver and adrenal glands.

In a parallel experiment mean forebrain ascorbic acid in deficient animals was, on day 15, 0.44 ± 0.05 μmol g^{-1} ($n = 4$), and, on day 42, 0.26 ± 0.25 μmol g^{-1} ($n = 4$). These values represent deficits, compared with controls (Table 1), of 60% and 77%, respectively. Brainstem showed deficits of 69% at 15 d and 63% at 42 d. On day 68 (Table 1) the deficits were 73% in forebrain, 71% in brainstem, 83% in liver and 86% in adrenals. It can be inferred that the brains of the deficient group were severely lacking in ascorbic acid both during maze learning and for at least 30 d before.

The chronic, severe brain vitamin C deficiency had no effect on T-maze performance, as assessed by either reversals or errors during 20 d of testing (Table 1). This indicates that learning is not enhanced by having saturating levels of ascorbic acid in the brain.

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Similarity of morphine abstinence signs to thermoregulatory behaviour

THE precipitated abstinence syndrome consists of a series of behavioural events which appear in morphine-dependent organisms after the administration of narcotic antagonists. Martin[1] has suggested that some precipitated abstinence signs arise because an error force is generated when a narcotic antagonist resensitises a homeostat previously altered by a narcotic. Recently, in studies on the neuroanatomical correlates of morphine withdrawal, we found that brain areas associated with the wet shake behaviour of precipitated abstinence in the rat seemed to be closely adjacent to central pathways of heat dissipation and heat gain[2–4]. As morphine has complex effects on central thermoregulatory mechanisms[5], the possibility was considered that the generation of an error signal in central thermoregulatory systems may account for the appearance of some precipitated abstinence signs. To test this hypothesis, we investigated the effects of environmental temperature on the precipitated abstinence syndrome.

Male Sprague-Dawley rats, obtained from Horton Laboratories (Oakland, California) and weighing 235 ± 21 g (s.d.) each, were used throughout these experiments. To produce dependence, two morphine pellets, each containing 75 mg of morphine base[6], were implanted subcutaneously in the lower abdominal wall. The pellets of morphine were implanted 48 h apart. Twenty-four hours after the last pellet implant animals were weighed, placed in glass jars and then randomized into three groups. One group was kept at room temperature (22 to 24°C). The other groups were transferred to a hot (34 to 37°C) or cold (6 to 10°C) room. After 1 h the abstinence syndrome was precipitated in these different ambient temperatures by injecting naloxone hydrochloride intraperitoneally. (Endo Laboratories, Garden City, New York). Withdrawal behaviour was observed continuously for 10 min, as previously described elsewhere.[7,8].

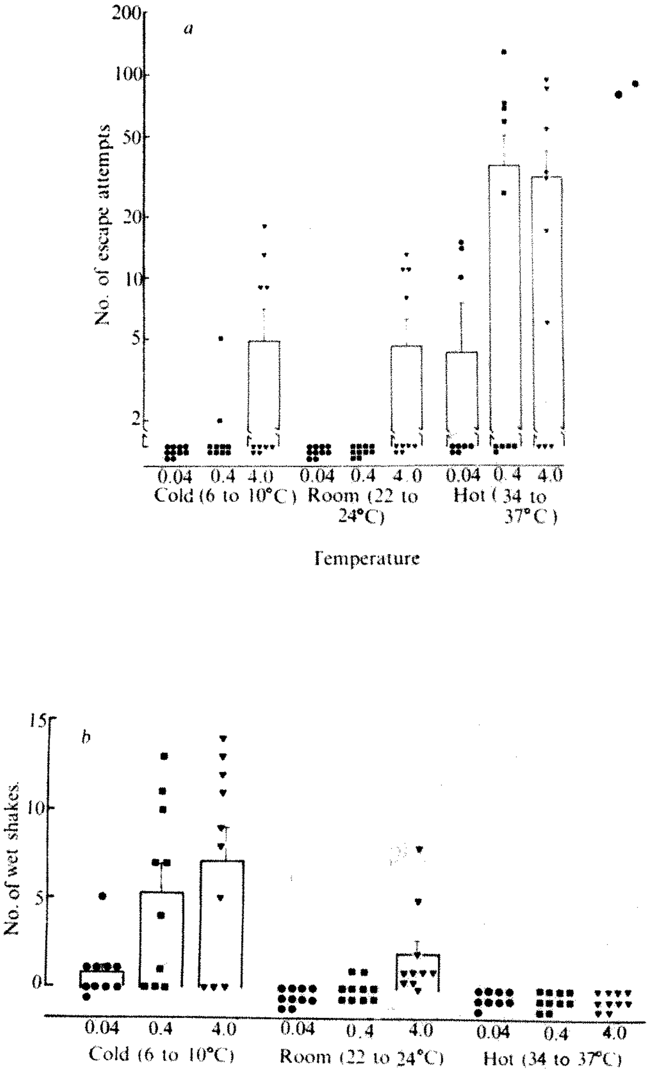

FIG. 1 Abstinence behaviour at different ambient temperatures. *a*, Escape attempts; *b*, wet shakes. Two pellets containing 75 mg of morphine base were implanted 48 h apart in male Sprague-Dawley rats weighing 235 ± 21 g (S.D.). Three days after the first pellet withdrawal was precipitated at different ambient temperatures by injecting naloxone hydrochloride at 0.04, 0.4, or 4.0 mg kg^{-1} intraperitoneally. Withdrawal beahaviour was observed for 10 min after naloxone administration. Each point represents the datum from one rat.

Animals placed in jars at the three different temperatures did not manifest any shaking behaviour or escape attempts. After 1 h in a hot environment, the ears and tails of the rats became red, the animal lay on its side with an extended posture and some salivation was observed. These signs have been reported as being characteristic behaviour of rats acutely exposed to a hot environment[9]. Animals kept in the cold, on the other hand, maintained a huddled position, the ears were blanched, and some degree of ptosis was observed.

As expected, the injection of naloxone in morphine-dependent rats kept at room temperature provoked the abstinence signs of wet shakes and escape behaviour[7,8]. The effect of a cold or hot environment on these withdrawal signs was dramatic. Escape behaviour, precipitated by naloxone in a hot environment, was markedly enhanced: jumping was elicited in some animals with a dose of naloxone as low as 0.04 mg kg^{-1} and at 4 mg kg^{-1} most animals repeatedly jumped out of the jars as fast as the observer was able to put them back into the container (Fig. 1*a*). The most active rat jumped out of its jar 132 times within a 10 min period. By contrast, wet shakes behaviour was completely suppressed in the hot environment (Fig. 1*b*). In the cold environment, the frequency of wet shakes was enhanced but no change in the escape attempt frequency was noted (Fig. 1*b*).

As a hot or cold environment will trigger vascular adjustments and alter metabolic rate, it could be argued that the behavioural effects of temperature were secondary to changes in the distribution of naloxone to the brain. The results in Table 1 indicate, however, that a change in ambient temperatures does not significantly affect, within the 10 min observation period, the distribution of naloxone in serum or brain.

The results of this study demonstrate that the occurrence of certain withdrawal signs, wet shakes and escape attempts, can be markedly influenced by environmental temperature. In this context, one may ask: what is the physiological significance of wet shakes and escape attempts in the behavioural repertoire of the normal rat and, why are these withdrawal signs magnified or suppressed by environmental temperature?

TABLE 1 Effect of temperature on the distribution of naloxone in serum in brain

Treatment	Naloxone levels Serum ($\mu g\ ml^{-1}$)	Naloxone levels Brain ($\mu g\ g^{-1}$)	Brain/serum
Placebo (22 to 24°C)	1.38 ± 0.09	0.76 ± 0.05	0.55
Morphine (22 to 24°C)	1.48 ± 0.21	0.69 ± 0.21	0.47
Morphine (6 to 10°C)	1.54 ± 0.16	0.86 ± 0.15	0.56
Morphine (34 to 37°C)	1.35 ± 0.25	0.76 ± 0.18	0.56

Rats were implanted with two placebo pellets or two pellets containing 75 mg of morphine base. The second pellet was implanted 48 h after the first pellet. Three days after the first pellet, two groups were transferred for 1 h to a cold (6 to 10°C) or hot (34 to 37°C) environment. Naloxone hydrochloride, at a specific activity of 1.9 μCi mg^{-1}, was administered 4 mg kg^{-1}, intraperitoneally, and 10 min later, serum and brain tissues were obtained and measured for naloxone. Five animals were in each group. Values represent the mean ± s.e. No significant differences were observed in the different groups.

Hainsworth[9] has observed that normal rats placed in a 40°C environment for 2 h or a 44°C environment for 1 h will try to escape from its cage by biting at any opening, by jumping up the side of the cage or by trying to push the top off the cage. By contrast, normal rats placed in extreme cold will demonstrate wet shakes. For example, cold water, wetness, and irritation to tissue around the ears will provoke this shaking behaviour[4]. It seems, therefore, that escape attempts and wet shakes, in certain experimental conditions, are forms of adaptive behaviour which enables the rat to maintain normal body temperature. Escape attempts could be a heat loss mechanism: the animal 'feels too warm' and attempts to escape from its environment. Wet shakes could be a heat gain mechanism similar to shivering.

Body temperature fluctuates widely during withdrawal from morphine[10-12]. Some of the signs of abstinence in man and in experimental animals may reflect this disruption of thermoregulation. For example, shivering, gooseflesh, sensations of cold and warmth, profuse sweating, rhinorrhoea and lacrimation are characteristic withdrawal signs in man[13]. In the rat, during precipitated withdrawal at room temperature, teeth chattering, wet shakes, vasoconstriction, ptosis and a huddled posture may be observed[7]. This behaviour may reflect activation of heat gain mechanisms. Other withdrawal signs are salivation, the spreading of saliva on the body and escape behaviour. These latter signs may be manifestations of heat loss mechanisms[9].

In recent studies we have presented data indicating that naloxone acts principally in medial mesodiencephalic regions of the brain to precipitate the abstinence syndrome[2-4]. The medial mesodiencephalon has traditionally been considered to be an important brain area for the integration of thermosensory information and for the regulation of body temperature[14]. Thus, from an anatomical viewpoint, it would seem plausible that certain precipitated withdrawal signs resemble a derangement of central thermoregulatory mechanisms. Escape behaviour and wet shakes may be viewed as behavioural events triggered by the sudden occurrence of an error signal in the thermosensory or thermal control system: compensatory heat gain and heat loss mechanisms are then activated to return the set-point to normality.

From these considerations, a tentative generalisation may be proposed for interpreting the effects of some drugs which modify the withdrawal signs of wet shakes or escape attempts. It is proposed that drugs which make the animal 'feel hot' will increase the frequency of escape behaviour and, possibly, suppress wet shakes whereas drugs which make the animal 'feel cold' will enhance the frequency of wet shakes without affecting escape behaviour. As pharmacologic agents frequently affect more than one component of the thermoregulatory system[15,16], considerable caution should be exercised in the interpretation of drug effects on morphine dependence[17]. The results of this study also indicate that it would be prudent to monitor environmental temperature during the observation of the withdrawal syndrome.

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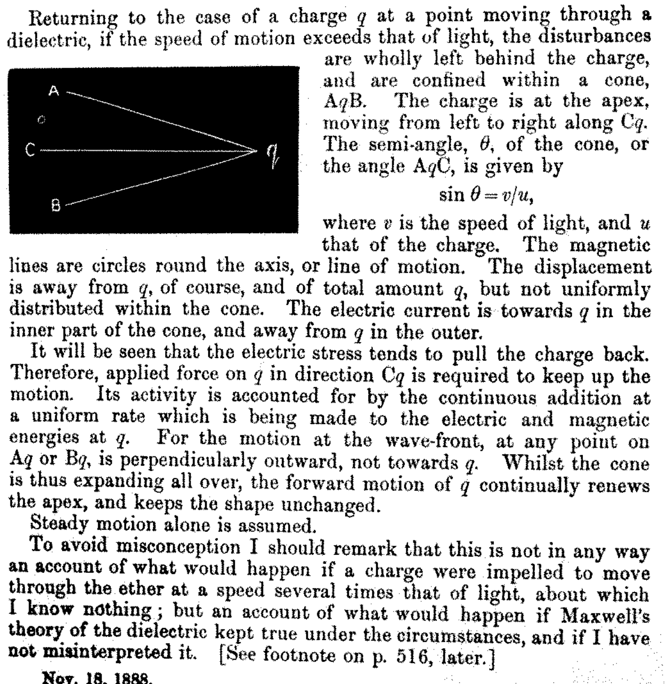
Heaviside Radiation

It is now well known that a charged particle, moving through a refracting medium at a speed in excess of the phase velocity of electromagnetic waves, produces radiation. The effect is observed with energetic electrons in water and other media, from cosmic ray particles moving through the atmosphere and may also be a source of very low frequency radio waves in the ionosphere and magnetosphere. Its discovery is usually attributed to Čerenkov[1] and its theoretical interpretation to Frank and Tamm[2], all three of whom were jointly awarded a Nobel Prize in 1958.



Returning to the case of a charge q at a point moving through a dielectric, if the speed of motion exceeds that of light, the disturbances are wholly left behind the charge, and are confined within a cone, AqB. The charge is at the apex, moving from left to right along Cq. The semi-angle, θ, of the cone, or the angle AqC, is given by

$$\sin\theta = v/u,$$

where v is the speed of light, and u that of the charge. The magnetic lines are circles round the axis, or line of motion. The displacement is away from q, of course, and of total amount q, but not uniformly distributed within the cone. The electric current is towards q in the inner part of the cone, and away from q in the outer.

It will be seen that the electric stress tends to pull the charge back. Therefore, applied force on q in direction Cq is required to keep up the motion. Its activity is accounted for by the continuous addition at a uniform rate which is being made to the electric and magnetic energies at q. For the motion at the wave-front, at any point on Aq or Bq, is perpendicularly outward, not towards q. Whilst the cone is thus expanding all over, the forward motion of q continually renews the apex, and keeps the shape unchanged.

Steady motion alone is assumed.

To avoid misconception I should remark that this is not in any way an account of what would happen if a charge were impelled to move through the ether at a speed several times that of light, about which I know nothing; but an account of what would happen if Maxwell's theory of the dielectric kept true under the circumstances, and if I have not misinterpreted it. [See footnote on p. 516, later.]

Nov. 18, 1888.

Fig. 1

In view of the extract shown in Fig. 1 (ref. 3), may I suggest that the name of the British scientist, Oliver Heaviside, should be associated with this phenomenon.

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Heaviside–Mallet Radiation?

FOR those who have worked on Čerenkov radiation it is of great interest to read that Kaiser[1] has unearthed a prediction of the effect by Heaviside that predates Čerenkov's early work by 46 years, and it is remarkable that apparently, this has not been pointed out before.

It would seem, however, at least from the extract quoted by Kaiser, that Heaviside did not suggest or carry out any experiments. Indeed this was virtually impossible at that time, as his paper predates the discoveries of cosmic rays and radioactivity, and there were, of course, no beams of artificially accelerated relativistic particles available at that time.

It is therefore on the theoretical side that Heaviside's work is significant and his name should thus be linked with those of Vavilov[2] and of Frank and Tamm[3].

Here I point out that, as far as the experimental side is concerned, it would seem rather that the credit should go to the French physicist Mallet, who, through his observations between 1926 and 1928 (refs. 4 to 6), was probably the first to study the effect always attributed to Čerenkov, a point already raised by myself[7], by Mallet himself[8] and by Perrin[9]. I therefore suggest that, for those who feel that changing established names for well known phenomena is justifiable, the title Heaviside–Mallet radiation might be more appropriate than the one put forward by Kaiser. In connection with double-barrelled titles it is of interest to note that for many years the effect has also been known as Vavilov–Čerenkov radiation, at least within the Soviet Union.

It is not inappropriate that a French name should be associated with the effect since there is little doubt that the glow from the concentrated radioactive solutions prepared by Mme Curie[10] was generated by the same mechanism; her observations took place around 1910.

This letter should not, however, be interpreted as discrediting three brilliant Nobel Prize winners, for by far the greater proportion of all theoretical work in the field has nevertheless been carried out by Russian physicists. There are sixty-two Russian references in ref. 7 alone and two other relevant sources of information, both Russian, are the treatises by Bolotovskii[11] and by Zrelov[12].

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Alternative to Holography for Determining Phase from Image Intensity Measurements in Optics

IN light optics and electron optics one normally measures the image intensity and attempts to infer from these measurements the object structure. In light optics these intensity measurements are insufficient to evaluate the detailed structure of the object and information on the phase of the transmitted light is of prime importance[1]; the interaction of the light or electrons in transmission through the object can be only satisfactorily explained by wave optics. The phase problem in optics is analogous to the X-ray diffraction phase problem, that is, whereas an object can be reconstructed from the phase information (even with incorrect amplitudes), it is not possible to define an object from amplitude measurements only[1]. We suggest here how a normal optical system can be used to determine both the amplitude and phase from image intensity measurements in bright-field optics. In bright-field optics, where the main (on-axis) beam is allowed to interfere with the scattered wave, the transmitted object wavefunction $\psi_0(r_0)$ can be written as

$$\psi_0(r_0) = 1 + \psi_s(r_0) \qquad (1)$$

where $\psi_s(r_0)$ represents the effect of the object on the incident light or electron wave at the point $r_0 = (x_0, y_0)$ in the object plane; ψ_s carries information not only on the amplitude attenuation of the wave but also phase shifts introduced by refractive index differences (potential differences in electron optics) across the object. The 1 represents the amplitude of the transmitted wave that is not scattered in the object, and this unscattered contribution will give a background in the image. The wavefunction $\psi_0(r_0)$ transmitted by the object may be subsequently affected by lens defects and restricting apertures in the optical system, particularly in electron optics, but for simplicity we shall assume that the wave at the image plane $\psi_i(r_i)$ is equivalent to $\psi_0(r_0)$. We record not $\psi_i(r_i)$ but the image intensity $|\psi_i(r_i)|^2 \equiv |\psi_0(r_0)|^2$; the image intensity is from equation (1)

$$\begin{aligned} j(r_i) &= |\psi_i(r_i)|^2 = |1 + \psi_s(r_i)|^2 \\ &= 1 + 2Re[\psi_s(r_i)] + Re^2[\psi_s(r_i)] + Im^2[\psi_s(r_i)] \end{aligned} \qquad (2)$$

where Re and Im denote the real and imaginary parts of ψ_s. At best, if the interaction between the light or electron wave and the object is small and the squared terms in equation (2) can be neglected, we can only determine the real part of ψ_s from image intensity measurements, and there is no direct way of evaluating the imaginary part of ψ_s, hence determining both the amplitude and phase. But instead of using a normal circular aperture in the back focal plane (Fourier plane) of the objective lens, we can use a semicircular (half-plane) aperture to cut off one-half of the Fourier plane of the object wavefunction. The Fourier plane, which corresponds to the diffraction or scattering plane, displays the spatial frequencies of the object structure, and the idea is to eliminate initially one-half of the object frequencies from the image. Now there is an explicit relationship between the real and imaginary parts of ψ_s. We maintain the bright-field situation by having a small centre section cut out from the aperture to allow the main beam through to interfere with the scattered wave—this is strictly necessary in electron optics to avoid electrical charging problems. Suppose we omit the negative half of the Fourier plane (spatial frequencies $\nu_x < 0$), but allow all positive frequencies to contribute to the image; then the image intensity corresponding to this situation (Fig. 1*a*) is, omitting subscripts i,

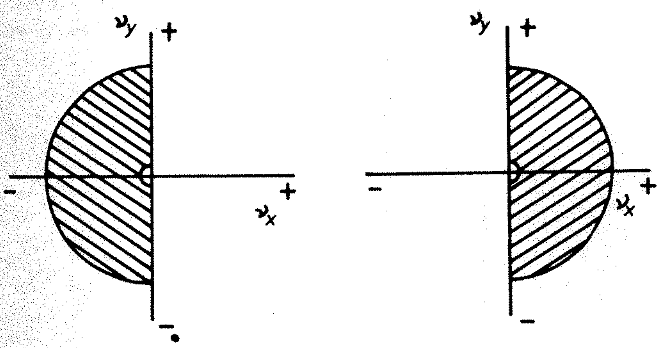

Fig. 1 Semicircular aperture positioned in the back focal (Fourier) plane of the objective lens. *a*, Negative half of plane excluded; *b*, positive half of plane excluded by the aperture.

$$[j(r)]_{\nu_x>0} = 1+2Re[\Psi_s(r)]_{\nu_x>0} + Re^2[\Psi_s(r)]_{\nu_x>0} + Im^2[\Psi_s(r)]_{\nu_x>0} \quad (3)$$

The idea of using a half-plane aperture is not new[2], but the important relationship between $Im(\psi_s)_{\nu_x>0}$ and $Re(\psi_s)_{\nu_x>0}$ has not been given. The relationship between the real and imaginary parts of $(\psi_s)_{\nu_x>0}$ can be derived from standard Fourier theory[3] and the analyticity of ψ_s[4], and in order to avoid detailed mathematics, we state the result that the imaginary part of $(\psi_s)_{\nu_x>0}$ is a one-dimensional Hilbert transform of the real part; that is,

$$Im[\psi_s(x,y)]_{\nu_x>0} = \frac{1}{\pi}\int_{-\infty}^{+\infty} \frac{Re[\psi_s(z,y)]_{\nu_x>0}}{z-x}\mathrm{d}z \quad (4)$$

taking the Cauchy principal value of the integral. From an image recorded using a semicircular or half-plane aperture we have an explicit relation between $Re(\psi_s)_{\nu_x>0}$ and $Im(\psi_s)_{\nu_x>0}$. But the squared terms in equation (3) prevent a direct calculation of $Re(\psi_s)_{\nu_x>0}$ from the image intensity $j_{\nu_x>0}$. We have solved this problem by initially neglecting these squared terms and we calculate $Re(\psi_s)_{\nu_x>0}$ from $1/2(j_{\nu_x>0}-1)$; using this approximation to $Re(\psi_s)_{\nu_x>0}$ we evaluate $Im(\psi_s)_{\nu_x>0}$ using a form of equation (4) which avoids the problem of the singularity at $z=x$ and also takes account of the finite extent of the actual image; again this is a mathematical detail. We now have values for both the real and imaginary parts of $(\psi_s)_{\nu_x>0}$ and a correction is applied to equation (3) for the squared terms, Re^2+Im^2; it may be necessary to apply this correction several times, depending on the relative contributions of the squared and linear terms to the image intensity $j_{\nu_x>0}$. In numerical tests, contributions from these squared terms of 25% of the value of $j_{\nu_x>0}$ can be systematically corrected in 1-5 iterations. This magnitude for the correction is well within the normal conditions of electron microscopy, where image contrast is only 20% (of which squared terms contribute 10%); $Re^2+Im^2=0.25$ (relative to the unity of the unscattered beam) would correspond to a very thick object with a strong interaction between the incident wave and the object, corresponding to about 25% scattering from the incident beam.

By using such a half-plane aperture we have excluded all the information from the negative spatial frequencies ($\nu_x<0$) and the result for just $(\psi_s)_{\nu_x>0}$ is not a complete representation of the object ψ_s (including all spatial frequencies). We avoid this limitation by using a complementary semicircular aperture (Fig. 1*b*) or by just inverting the first half-plane aperture, excluding the $\nu_x>0$ half of the Fourier plane, to give an equation corresponding to (3) with $\nu_x<0$; the Hilbert transform relationship between $Re(\psi_s)_{\nu_x<0}$ and $Im(\psi_s)_{\nu_x<0}$ may again be derived, differing from (4) only in a change in sign on the right-hand side of the equation. Thus by recording two images with complementary semicircular (half-plane) apertures (Fig. 1) we can reconstruct from image intensity measurements the scattered wave immediately after the object ψ_s, by the addition of the two half-plane functions $(\psi_s)_{\nu_x>0}$ and $(\psi_s)_{\nu_x<0}$.

If the object structure has a centre of symmetry or a mirror plane we need only one image taken with a semicircular aperture to determine the complete object wavefunction ψ_s.

There are practical points to be considered if this method is to work; for example, the two component (half-plane) images of ψ_s, $(\psi_s)_{\nu_x>0}$ and $(\psi_s)_{\nu_x<0}$, have to be added to give ψ_s and the problem of correlating two images arises[5]. It is assumed that during the period of recording the two images the object does not change (for example, due to radiation damage in electron optics), that the objective lens focus does not change, that the two apertures are exactly complementary. The experimental arrangement seems fairly simple in light optics but the electron-optical equivalent, in which the sizes of apertures are scaled down to 50 μm, is subject to the problems of severe contamination and electrical charging of the half-plane aperture[6].

The method outlined here for determining the amplitude and phase from image intensity measurements does not have the non-uniqueness problems associated with general iterative schemes for determining the phase[7,8], because we have a very good approximation to the real part of the wavefunction and we derive a value for the imaginary part which should at least place the phase angle ($\tan^{-1}(Im/Re)$) in the correct quadrant. In bright-field optics we propose that the method of recording complementary half-plane images can be used to determine the amplitude and phase of the object wavefunction, without severe restrictions on the magnitude of the wavefunction, such as the phase grating approximation. The method does not work in dark-field optics, where the main (unscattered) beam is excluded from the image; this is because we can no longer derive an approximation to $Re(\psi_s)$ as in the bright-field case and the iterative solution of the Hilbert transform equation (4) is no longer unique.

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Acuity and contrast sensivity of infant vision

If we are to study the development of form and space perception in man, it is necessary to know something of the basic visual capabilities of the developing human infant. There have been a number of investigations of visual acuity in the first few months of life. Using fixation preference as a measure, Fantz *et al.*[1] showed that infants could discriminate a stripe pattern of 1.5 cycles per degree by 1 month, and 3 cycles per degree by 2 months. Dayton *et al.*[2] found that newborn infants showed optokinetic nystagmus to a 4 cycles per degree pattern. These studies demonstrate a useful degree of visual spatial resolution in very young infants, though one markedly poorer than the typical adult value of 40 cycles per degree.

An observer's visual acuity is a measure of the highest spatial frequency (finest grating) that he can detect. Much more information about the spatial performance of the visual system is expressed by a contrast sensitivity function[3,4]; that is, the threshold contrast for a range of sinusoidal gratings that vary in spatial frequency. We report here a preliminary attempt to determine the contrast sensitivity function of an infant, who was aged 50–62 d when the observations were made.

We used Teller's[5] modification of the fixation preference technique. The infant was held in front of a display with two windows. On each trial one window contained a vertical grating while the other contained a uniform field of equal mean luminance. One experimenter observed the infant from behind the display, without knowing which window contained the grating. On the basis of 15 s observation of the infant's fixations and other behaviour, he had to guess on which side the grating was presented. For a given spatial frequency, the trials were run in blocks of five of constant grating contrast, the contrast being varied between blocks according to a modified staircase procedure[6] (average length of staircase, 50 trials) until the value of contrast at which the observer gave 70% correct reports could be estimated. This value was taken as a 'threshold' contrast for that frequency. The procedure was then repeated for a different frequency. The validity of the technique is supported by the finding that the % of correct reports declined monotonically with decreasing contrast when frequency was held constant. For comparison, we also measured the contrast threshold of an adult observer in the same apparatus by the method of adjustment, at each frequency used.

The gratings were produced by the sinusoidal modulation of a high-frequency raster on a large oscilloscope screen[3]. The modulating oscillator was gated so that the grating appeared in one half of the screen only, that is, behind one of the windows, the unmodulated raster appearing behind the other. The grating was presented to left or right on successive trials according to a random sequence. Contrast was varied by passing the oscillator output through an attenuator. The mean luminance of both windows was 2.2 cd m^{-2}. At the infant's viewing distance (57 cm), the square windows subtended 7.5° each and their centres were separated by 18°.

Refractive examination of the infant showed that she required no spherical correction but showed some astigmatism (left eye +1D, 70°; right eye +0.5D, 90°). The ocular media and retina appeared normal. The stimuli were thus within the probable accommodative range of the infant[7]. It was quite possible, however, that she was not correctly accommodating; this adds to the grounds for regarding the obtained data as a lower limit on her discriminative capacities.

There were two types of presentation. In one, the gratings drifted across the window at 4.7 degrees s^{-1}, the direction of motion reversing every second. In the other, the modulation was stationary but was switched on and off ('flashed') at 1.5 Hz, mean luminance remaining constant.

The results for the infant and adult observers, for both types of presentation, are plotted in Fig. 1. The infant's sensitivity is markedly depressed overall compared to the adult's; however, it should be remembered that we depended upon an observable preference to indicate the infant's detection, and so her results can be regarded only as giving a lower limit on her capacity for detection.

The infant's sensitivity functions show a number of features found in the adult. In particular, both infant and adult had a peak sensitivity for flashing stationary gratings at intermediate frequencies with a sharp high-frequency cut above 4 cycles per degree, and a decreasing sensitivity below 1 cycle per degree.

Both showed better performance with flashing than with drifting gratings at high spatial frequencies, and a more marked low-frequency cut with the flashing grating[8]. The extent of the low- and high-frequency cuts with the drifting display was presumably due to the use of a constant drift velocity (a temporal modulation rate proportional to spatial frequency). For the adult, the peak sensitivities for the two types of display were equal, but the infant showed greater overall sensitivity for flashing gratings. We cannot say whether this is due to a true difference in relative detectability, or to a more marked preference for flashing gratings than for drifting gratings of equal detectability.

Above 1 cycle per degree, the infant's curves are each depressed by a constant ratio of contrast relative to the adult's. This depression is greater for the drifting than for flashing gratings. At the low spatial frequencies, however, the

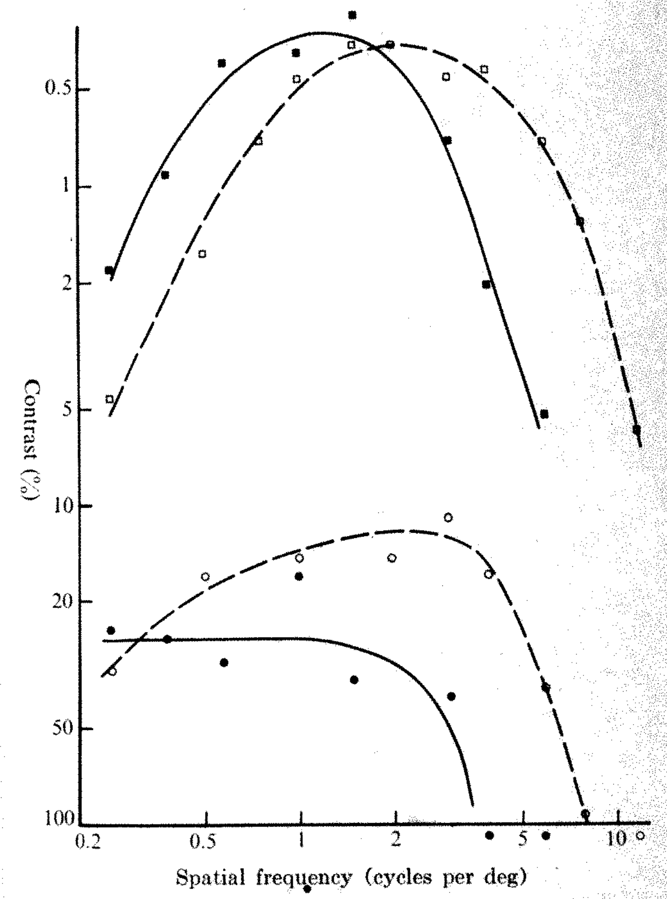

Fig. 1 Contrast sensitivity as a function of spatial frequency, for infant and adult observers (see text for methods of measurement). ■, □ Adult observer; ●, ○, infant observer; ○, □, flashing gratings; ●, ■, drifting gratings. Both axes are logarithmic, and the ordinate is plotted with contrast decreasing upwards. Points below the abcissa indicate values of spatial frequency for which discrimination did not reach the 70% level for 100% contrast.

gap narrows for both curves; the infant appeared relatively more sensitive in this region. Again, the difference can be explained in two ways. The visual pathway of the infant may be so organised as to transmit low spatial frequencies relatively well; for example, her visual neurones may have receptive fields whose inhibitory receptive fields are less developed than their excitatory centres. Alternatively, the difference may be due to a preference for low spatial frequencies over equally detectable high frequencies.

The point at which the curves cut the abcissa (100% contrast) represents visual acuity as conventionally measured with high-contrast targets. For the infant viewing stationary flashing gratings this point is at about 8 cycles per degree. This is a higher acuity than that previously found for infants 60 d old and younger. This can be attributed to the use of forced-choice by a 'blind' observer, which allows the use of more varied and subtle aspects of the infant's behaviour than simply timing fixations, and to the use of drifting and flashing targets, which are probably more attention-compelling to the infant than the static patterns used in earlier preference studies.

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Heavy water nystagmus and effects of alcohol

AFTER drinking a large dose of ethyl alcohol (1 or 2 g of alcohol kg^{-1} of body weight), a phenomenon called positional alcohol nystagmus (PAN) occurs[1,2]. Nystagmus is an involuntary oscillating movement of the eyeballs in which they repeatedly turn slowly in one direction and fast in the reverse direction. The nystagmus that starts 30 min or so after drinking alcohol (PAN I) is called positional because it occurs only when the head is held in certain positions relative to gravity. When the head is held with the right side down, the eyes turn (with a frequency of roughly two complete oscillation s^{-1}) fast to the right, slowly to the left, fast to the right, and so on. Nystagmus is named after the prominent fast movements, and this nystagmus is called nystagmus 'to the right'. When the head is held with the left side down after alcohol, the resulting nystagmus is to the left.

It has been known for more than 40 yr (ref. 1) that PAN is caused, after ingestion of alcohol, by the action of gravity on the vestibular apparatus. In people or other animals who have lost the vestibular apparatus, the organ of balance, PAN never occurs. It was always thought puzzling that these vestibular sensory receptors could initiate a gravity-dependent nystagmus, because standard theory[3] holds that the two otolithic receptors are sensitive to gravity but cannot cause nystagmus, whereas the three semicircular canal receptors are not sensitive to gravity but do cause nystagmus. The mechanism whereby the vestibular receptors initiate PAN now seems obvious in the light of 'heavy water nystagmus', described here.

In ten male human subjects who had been fasting at least 10 h, we observed eye movements by using Frenzel spectacles and by using electrooculography[2] behind closed eyelids. Eye movements were observed with the subject's head in the supine position, with the left side down, and with the right side down, and the tests were repeated hourly between 30 min and 7½ h after drinking heavy water (deuterium oxide). The subjects weighed between 67 and 86 kg, and the dose was not adjusted for body weight; five subjects drank 200 g of deuterium oxide and five drank 100 g.

None of the subjects had spontaneous or positional nystagmus before drinking deuterium oxide, and one subject had none after drinking 100 g of it. In four of five subjects after drinking 100 g and in all five after drinking 200 g, there was vigorous positional nystagmus in the head—lateral positions (Fig. 1). When the head was held with the right side down, the nystagmus was to the left; when the head was held with the left side down, the nystagmus was to the right. These nystagmuses were present 30 min after drinking deuterium oxide and they could be elicited for a further 5 to 6 h. Heavy water nystagmus has directions opposite to PAN 1.

Most of the subjects experienced strong sensations of bodily rotations, dizzyness, and nausea while lying in the head—lateral positions. The nausea subsided slowly between tests, and the other effects disappeared a few seconds after

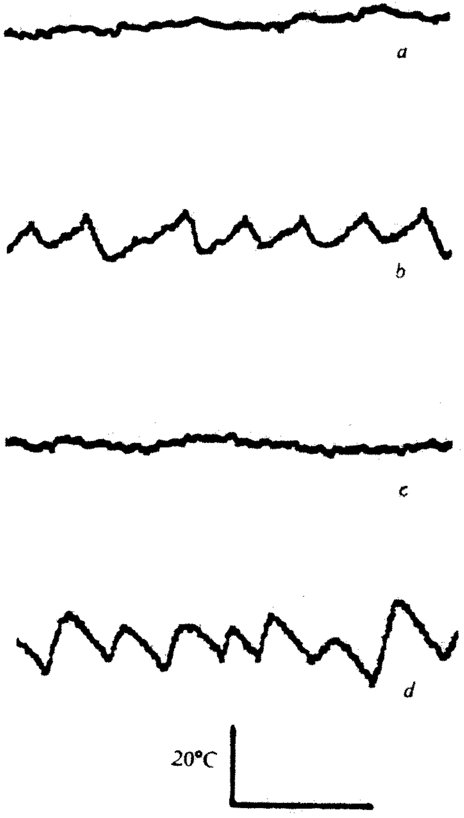

FIG. 1 Electronystagmographic traces of horizontal eye movements in a human subject with eyes closed. Pen movement upward indicates eye movement to the right. *a*, Lying right side down, before drinking D_2O; *b*, lying right side down, 30 min after drinking 200 g of D_2O; *c*, lying left side down, before drinking D_2O; *d*, lying left side down, 30 min after drinking 200 g of D_2O.

assuming the normal upright posture. It was not surprising that the motion sickness syndrome appeared during the tests, as it is well known[4] that unusual vestibular stimulation is the cause of motion sickness. There were no exceptions, in our limited sample of ten human subjects, to the generality that a subject's degree of nausea in the heavy water experiment varied inversely with his experience with alcohol ingestion. The heavy drinkers did not have nausea whereas the abstainers suffered greatly.

In a second experiment with four cats we demonstrated alcohol nystagmus and, after an interval of 1 week, we demonstrated heavy water nystagmus, using both electronystagmography in the dark and visual observation in the light. All of the cats after ingesting the deuterium oxide showed both the horizontal and rotary nystagmuses, all with directions opposite to those found[5,6] in the PAN I of cats. This result is consistent with the generality that heavy water nystagmus is precisely the opposite of PAN I. After a further interval of 1 week, heavy water and alcohol were administered in a mixture of the original doses of 4 ml of heavy water kg^{-1} and 2 ml of alcohol kg^{-1}, to see whether the presumed opposite effects would cancel. In two of the cats a weak nystagmus of the heavy water type was discernible briefly, and in the other two cats no nystagmus occurred; this result was in contrast to the vigorous and prolonged nystagmus in all four cats after ingestion of either chemical alone.

The apparent mechanism whereby heavy water elicits nystagmus is very simple. The cupula of the semicircular canal has the same density as the liquid (endolymph) in which it floats. The cupula is normally moved by circular endolymph movements resulting from angular accelerations, and because of its neutral buoyancy the cupula normally is not moved by linear accelerations or by gravity[7,8]. After ingestion of heavy water, the base of the cupula, because of its proximity to blood capillaries, acquires heavy water more rapidly than the surrounding endolymph. The cupula thereby loses its neutral buoyancy sufficiently[8-10] so that gravity can move it.

Alcohol nystagmus can be similarly explained. In cats with various semicircular canals discretely inactivated it has been shown[5,6] that, in PAN, gravity acts on the semicircular canals. This finding concerning the site of action of gravity has been independently confirmed[11], but it has not been explained how the canals become sensitive to gravity after alcohol ingestion. It now seems obvious that, just as heavy water can make the cupula heavier than endolymph until the heavy water diffuses well into endolymph, so alcohol which is lighter than water can make the cupula lighter than endolymph until the alcohol concentration in endolymph rises sufficiently.

A variety of implications follows from these findings and interpretations. The second phase[12,13] of alcohol nystagmus (PAN II) probably results from the presence of alcohol in endolymph after the alcohol has largely disappeared from the cupula, and residual alcohol in the inner ear (with resultant symptoms of motion sickness) is probably a factor in the production of a hangover. In the clinical and experimental uses of heavy water for determination of total body water, the head—lateral orientations should be avoided because motion sickness and a profound antidiuresis[14] can result.

Anyone who has assimilated heavy water, even as little as 45 ml through the lungs and skin, should avoid circumstances where bodily disequilibrium would be disastrous. Although it is a standard clinical view[15] that non-fatiguing positional dizzyness and nystagmus are usually caused by the action of gravity on the utricular otoliths (often in the presence of central pathology), it now seems possible that some of these conditions are caused simply by the cupula's loss of neutral buoyancy.

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Temperature distribution inside a burning cigarette

THE study reported here has been undertaken to resolve the large discrepancies between reports of the temperature distribution in the combustion coal of a cigarette. Studies using bare thermocouples (refs 1–4 and R. G. Hook, paper presented at Twentieth Tobacco Chemists' Conference, Winston-Salem, North Carolina, November 1966) indicate that the highest temperatures occur on the central axis of the coal (the actual reported values vary considerably but are usually in the range 800–900° C during a puff, 700–800° C during the natural smoulder between puffs, and 800–850° C under steady state continuous draw conditions). Under all smoking regimes, the thermocouple-measured temperature decreases by up to 300° C along a radius from the maximum central temperature to the periphery of the coal, although one thermocouple study[2] has reported the occurrence of occasional peripheral hot spots, with temperatures higher than the centre of the coal. (Ref. 2 contains a comprehensive compilation of more than forty studies of temperature measurements inside burning cigarettes prior to 1968.) In contrast, temperature measurements by X-ray observations of the melting of small metal particles placed within the cigarette[5] indicate that the highest temperatures (900° C) occur at the periphery of the coal. Furthermore, measurements of the coal's surface temperature by radiation methods (ref. 5 and A. T. Lendvay, F. M. Watson III, and T. S. Laszlo, paper presented at the CORESTA/Tobacco Chemists' Joint Conference, Williamsburg, Virginia, October 1972) have recorded temperatures of about 850–920° C during a puff, 700° C (below the surface ash) during smoulder, and short-lived hot spots on the coal's surface as high as 1200° C (ref. 5).

In all previous studies, no attempt has been made to define exactly which temperature is being measured in the heterogenous coal—that of the solid matrix of the coal or that of the gases and smoke within the coal. Bare thermocouple junctions, and the metal particles of the X-ray study, would be in physical contact with the tobacco strands and so would indicate a temperature intermediate between that of the gas and the solid phases, the actual temperature depending on the degree of strand-junction contact. On the other hand, radiation methods would indicate solely the temperature of the solid matrix.

In the present study, a quartz probe has been used to measure the gas- and solid-phase temperatures independently inside a cigarette. The probe contained a platinum/platinum –13% rhodium thermocouple junction (0.05 mm diameter wire) mounted at its end, and an infrared transmitting fibre optic (0.15 mm outer diameter, part of a Vanzetti 1074 thermal probe system). The fibre optic was sheathed with a metal tube for 6 mm to restrict its field of vision to directly ahead. Its viewing end was placed 12 mm behind the end of the quartz tube so that the delicate fibre optic was not subjected to the high ambient temperatures inside the coal. Non-filter English-type cigarettes (68 mm long, 8 mm diameter) were selected for weight (1.00 ± 0.02 g) and pressure drop (10.6 ± 0.3 mm water gauge, at a flow rate of 2.0 $\text{cm}^3\ \text{s}^{-1}$) and conditioned at 21° C and 60% relative humidity (1 mm water gauge = 9.8 N m^{-2}). The probe was inserted radially into the cigarette and the hole in the cigarette paper sealed with a minimum amount of latex solution. The cigarette was smoked at a constant draw of 2.0 $\text{cm}^3\ \text{s}^{-1}$. The temperature–distance relationships were determined at a fixed point, initially 20 mm from the lighted end of the cigarette, and distances of 0, 2, 3 and 4 mm from the central axis (4 mm is the surface measurement). The internal temperature profiles are the mean of four determinations whereas the external temperature profile is the mean of twenty determinations (because of the presence of variable surface hot spots).

The temperature distributions above 300° C are reported. Since smoke aerosol particles do not exist above about 300° C, the coal above this temperature is a gas-solid system. The contribution of radiation from heteropolar molecules to the overall infrared emission inside the coal is negligible. The temperature obtained from the radiation transmitted and detected by the fibre optic probe system (1.2–1.6 μm) is thus almost entirely that of the solid matrix of the coal (considered to be a blackbody). The thermocouple junction situated at the end of the quartz tube was positioned so that it was not in physical contact with tobacco strands, and so received heat from the gas phase by convective transfer and from the solid phase by radiation. Heat transfer calculations, using the solid phase temperatures obtained from the infrared system, together with the thermocouple temperatures, gave the temperature distribution of the gas phase. The analysis indicated that heat exchange with the gas phase is the predominant mechanism by which the thermocouple juction receives heat.

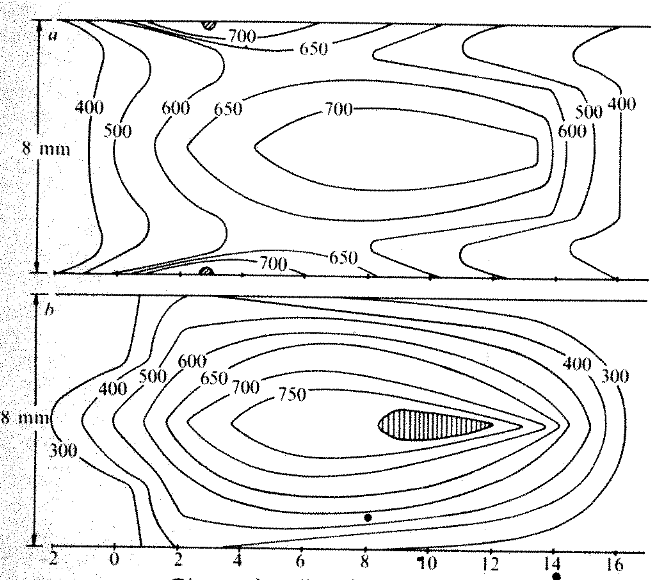

FIG. 1 Temperature distribution within the coal, with cigarette smoked at a constant draw of 2.0 $\text{cm}^3\ \text{s}^{-1}$. *a*, Temperature of solid (hatched area 750° C); *b*, temperature of gases (hatched area >800° C).

By analysing temperature values obtained in different experiments with the probe inserted radially and axially to given positions inside the cigarette (and hence subject to different temperature gradients), an empirical correction has been applied to the present results to compensate for heat losses by conduction along the probe and thermocouple leads. This correction can be as high as 96° C in the peripheral region of the coal, where the temperature gradients are steepest.

The distributions obtained for the temperatures of the two phases are shown in Fig. 1. They are not alike, indicating that thermal equilibrium between the gas and solid phases has not been achieved. The mean maximum solid temperature (750° C) occurs on the surface of the coal, at a distance of 2.6 mm in front of the line of paper burn. In addition, small hot spots of diameter 0.1–0.8 mm and temperature 780–830° C occur on the surface of the coal at variable distances of between 1 and 5 mm in front of the line of paper burn. Since an individual tobacco strand in the cigarette has a thickness and width of 0.1 and 0.5 mm respectively, these hot spots probably correspond to the behaviour of individual tobacco strands. For distances of less than 1 mm along any radius below the surface of the coal, the temperature of the solid decreases due to the occurrence of endothermic heterogeneous reduction of carbon dioxide by the carbonaceous coal, the occurrence of endothermic pyrolysis reactions and heat exchange with the cold gases entering the interior of the coal. At distances of more than 1 mm below the coal's surface (along any radius), the temperature of the solid phase starts to increase, and attains a temperature of 600–750° C over a large proportion of the interior of the coal.

In contrast, the gas phase temperatures at the coal's surface are relatively cool (≤400° C). Beneath the surface of the coal, and towards the cigarette central axis, the temperature of the gases continuously increases due to the occurrence of exothermic gas phase reaction (such as the oxidation of carbon monoxide) and heat exchange with the solid matrix. The maximum temperature of the gases in the coal (810° C) occurs on the central axis of the cigarette, 11.5 mm in front of the line of paper burn. In the interior regions of the coal (2 mm or more below the surface), the temperatures of the solid and gases are relatively close (to within 60° C), and dT/dr values for the two temperatures are similar and both have positive values (T = temperature, r = distance below surface of the coal). In this region of the coal, thermal equilibrium is closest to being attained.

This work has shown that, where heterogeneous reactions are occurring within porous beds, the phases involved can have widely differing temperatures, which may be determined by the techniques described.

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Inhabiting an island

Surtsey, Iceland: The Development of a New Fauna, 1963–1970: Terrestrial Invertebrates. By Carl L. Lindroth, Hugo Andersson, Högni Bödvarsson and Sigurdur H. Richter. Pp. 280. (Supplement 5 of *Entomologica Scandinavica.*) (Munksgaard: Copenhagen, October 1973.) 140 Dan. Cr.

THE volcanic island of Surtsey appeared in November 1963, had assumed its present form by July 1967, and is now the southwesternmost and second largest member of the Westman group. At the Surtsey Biology Conference in Reykjavik on May 28, 1965, it was decided to keep the progressive arrival of plants and animals on the new island under close observation: Dr Lindroth and his three colleagues were entrusted with the investigation of the terrestrial invertebrates.

The book is divided into three parts. In the first part the authors stress the advantages Surtsey has over similar new volcanic islands for this sort of study. They point out that the other small Westman islands were all formed by volcanic action about 6,000 years ago and probably acquired their fauna and flora stage by stage, in a way it is now possible to observe in detail on Surtsey, but that it may be centuries before the Surtsey biota reaches a climax comparable to that on the other islands.

In the second part of the book, the terrestrial invertebrates of Surtsey and its 'Hinterland' are listed, each with its local and general distribution and ecology. The term 'Hinterland' is used to include the other small Westman islands, Heimaey and the adjacent south coast of Iceland. Most of this part is devoted to exclusively Hinterland species but, as the authors explain, a knowledge of the numerous species which might have spread to Surtsey but have not yet done so is an essential part of their study. The 159 species actually recorded from Surtsey consist of 131 pterygote insects (of which 105 are Diptera), six Collembola, five spiders and seventeen mites.

In the third and largest part of the book, details of the frequency and abundance of the Surtsey species are given. Modes of dispersal, namely aerial (both active and passive), hydrochorous, zoochorous and anthropochorous, are discussed at some length. Precautions which are being taken to minimise anthropochorous dispersal and experiments to test the likelihood of hydrochorous dispersal are described. It is concluded that, although certain migratory Lepidoptera and one species of ballooning spider may have arrived direct from Europe or even North America, nearly all the species recorded from Surtsey have spread from the Hinterland. Most of the species require conditions for the completion of their life cycles which are not yet available on the island, largely because of the very slow spread of vascular plants; and the authors have found that, excluding insects breeding in carcasses washed up on the shore, only five species—one of Diptera, two of Collembola and two of mites—have become established by forming breeding populations. Finally, an attempt is made to predict the changes which are likely to take place in the fauna and flora of Surtsey during the next century.

The book contains numerous tables, diagrams, maps and photographs. Most of these are clear and informative but some of the photographs lack definition and the scale of some of the maps is rather too small. It is rather difficult to find one's way about the book owing to lack of clear distinction in the hierarchy of subheadings: centring of the earlier subheadings with the use of more distinctive lettering would have made the book easier to read.

But these small defects in presentation are relatively unimportant and Dr Lindroth and his co-authors are to be congratulated on producing an invaluable record of the initial stages of this unique natural experiment: the Surtsey Biology Conference was fortunate in its choice of such an able team. And owing to the recent devastating eruptions on Heimaey the detailed investigation of its fauna, though incidental to the main study, has its own special value.

E. H. EASON

Primates compared

An Atlas of Primate Gross Anatomy. By Daris R. Swindler and Charles D. Wood. Pp. 370. (University of Washington Press: Seattle and London, August 1973.) $30.

THE maturation of comparative primate biology as an academic field during the past fifteen years has seen a resurgence of interest in primate morphology. Such study has again become an active research field as well as a basis for the functional appreciation of primate fossils. In this context, much emphasis now attaches to the quantitative definition of variable morphological features that are functionally related to aspects of behaviour such as locomotion. Correspondingly, undergraduate training in physical anthropology includes instruction in the relevant basic comparative morphology.

Texts in this field suitable for use with honours students are not extensive and comprise, in general, accounts of only a single genus. This volume takes a welcome step in providing textual notes supplementing an extensive series of illustrations of high quality, grouped to form 149 plates. The whole is well set out to facilitate comparison between three primate genera: baboon (*Papio*), chimpanzee (*Pan*) and man (*Homo*). *Papio* and *Pan* were chosen for comparison with man because of their wide use in biological and clinical research and because they ". . . represent very different grades of locomotor adaptations" (*Papio*—a terrestrial quadruped; *Pan*—a brachiator, with *Homo*—a biped). Illustrations for the two subhuman genera are based on a series of dissections of twenty-two baboons and six chimpanzees; those for man are composite from numerous published sources.

The approach is regional although there are some allusions to systemic study. The result is a compilation which even although textually brief presents an unparalleled series of well drawn comparative figures. Each is intended to represent the norm of the series examined, certain inserts referring to principal variations.

Although proper attention is drawn to conspicuous topographical and proportional differences between the genera, the main impact of the text is to emphasise their basic similarity: this characteristic may well enhance the value of the volume as a student's text. Its potential worth as a reference monograph is, however, lessened by the fact that, despite the author's emphasis in the introductory sections upon the value of a proper appreciation of variation, its documentation is inadequate. Variation within and between these genera is marked; in some features, it can be readily defined as meristic; in others it is continuous and it is in relation to these that significance emerges in connection with functional contrasts.

The series of dissections on which the volume is based is, in many ways, unique, and the inclusion of relevant numerical data, even as an appendix, would give value as a research work to a volume which, in its present form, is basically only a teaching manual.

E. H. ASHTON

Ancient invertebrates

Paleobiology of the Invertebrates: Data Retrieval from the Fossil Record. By Paul Tasch. Pp. xxv+946. (Wiley: New York and London, August 1973.) £10.

THIS book is intended as an advanced textbook of invertebrate palaeontology, and is a very ambitious undertaking for a single author. It is comprehensive, detailed and profusely illustrated. Unfortunately, however, it gives the impression of having been written much too quickly for its thickness. Altogether it is a most exasperating book, despite its compendiousness, and it is not worth the price.

There are countless spelling mistakes. These are not trivial since, after all, zoological names need to differ by only one letter to be validly distinct. Thus, on page 159 alone, *Aulacophyllum* is written as *Aulocophylum*, *Phillipsastraea* as *Phillipastrea* and *Zaphrentis* as *Zaphrenthis*.

More serious are errors of fact. Nemerteans are never parasitic (contrast page 445). There is no reason why a type subspecies need occur earlier than a non-typical subspecies (page 853). Pogonophora are not a genus of hemichordate (page 810) and ascidians are not hemichordates either (page 635). The anal pyramid of eocrinoids had to do with defaecation, but this is not the same as excretion (page 709). The segments of an ophiuroid's arms are not the same thing as its vertebrae (page 736). The word "asterozoan", contrary to the muddled discussion on page 736, is not synonymous with "asteroid". Hedgehogs, indeed, have never been included in the echinoderms (page 631). Most of these mistakes are slips of the pen or due to stylistic slovenliness, but they have been printed nevertheless, and wait in ambush for the student.

The author has a pretentious habit of using 'cybernetic' terminology whenever he sees the opportunity: the questions at the end of each chapter are called "feedback", the bibliography is called "storage", the anatomy of a group comes under "encoded data" and its stratigraphy and palaeoecology under "derivative data". The advantages of this procedure escape me. On the other hand, such necessary words as "haemocoel" (page 313), "lobe" and "saddle" (page 386) and "metapinnule" (page 739) are used without explanation of what they mean.

There are several banal discussions on problems that are insoluble in principle, such as why groups died out. The last of the arthropleurids apparently became extinct "probably through failure to yield adequate numbers of viable offspring" (page 606). Parablastoids and edrioblastoids died out from a "continual decrease in the number of reproducing individuals" (page 725). But echinoids are ready for a great expansion in the future (page 695).

Concerning the index, I tried to look up the word '*Halycystus*', used on page 127, suspecting it might be identical with '*Haliclystus*', which happens to be the correct spelling, on page 129. After some search I found it, with the wrong spelling, under 'S' for 'Scyphozoa'. Is this the data retrieval referred to in the title?

It may be that a new advanced textbook of invertebrate palaeontology is now needed, but this book is not the one.

R. P. S. JEFFERIES

Glass

Glass Science. By Robert H. Doremus. (Series on the Science and Technology of Materials.) Pp. viii+349. (Wiley-Interscience: New York and London, August 1973.) £9.

THIS is an authoritative well written book in an area where relatively few books are available. The dust cover note says that this book "will be used as a text for advanced undergraduate and graduate level courses in glass science and by technologists of diverse backgrounds who are involved in the manufacture and use of glass in industry". The author himself states that his object is to describe the state of glass science in 1972, a much more satisfactory description of the scope of the book if one interprets the title to mean "the physics and chemistry of glasses". The title has some unfortunate suggestion of a special kind of science, a comment which the author tends to invite by his generosity in quoting opinions and hypotheses.

Readers of the book are expected to be conversant, for example, with X-ray diffraction and its interpretation by means of pair distribution functions, the Mössbauer effect, NMR, infrared and Raman spectroscopy, and solution thermodynamics. A copy of Morey's well-known book *Physical Properties of Glass*, long since out of print, unfortunately, is assumed to be available.

The book contains five parts; 100 pages on the formation and structure of glasses, 113 pages on transport processes of which 80 deal with diffusion and electrical conduction, 60 pages on chemical and surface properties, 40 on strength and 14 on optical properties. The choice of subjects reflects the author's own interests. Each of the eighteen chapters has on average sixty references to original papers so the book is an excellent entry to the literature. The author has succeeded in his aim of reviewing the information available in 1972.

The book will be especially useful to people who have recently taken up work in this field. It should certainly be on the reading list for advanced students of glasses.

R. W. DOUGLAS

Biological *bouchées*

Cell Differentiation. By J. M. Ashworth. Pp. 64. *Cellular Development.* By D. R. Garrod. Pp. 64. *Functions of Biological Membranes.* By M. Davies. Pp. 62. *Biochemical Genetics.* By R. A. Woods. Pp. 64. (Outline Studies in Biology.) (Chapman and Hall: London; Halsted (Wiley): New York, May 1973.) £0.90 each.

THE breakdown of the barriers between biological disciplines in university teaching has made it difficult to find adequate textbooks, for few reflect the broader approach now appropriate. A similar difficulty exists in providing suitable reading material for the increasing number of students who combine a variety of shorter courses in some form of integrated degree course. This new series of readers, each containing the equivalent of a short lecture course on a selected topic, could make a valuable contribution towards solving some of these difficulties and is therefore extremely welcome.

Ashworth, on *Cell Differentiation*, has selected a set of model systems that illustrate increasing levels of complexity from prokaryotic and eukaryotic organisms, and has shown how the basic behaviour can be analysed by genetic and biochemical techniques. He also gives an account of the characterisation (mainly biochemical) of such classic systems as chromosome structure in *Drosophila*, RNA synthesis in *Xenopus* and protein metabolism in different tissues, covering a wide range of experimental approaches. Ashworth contrasts the total analysis that is apparently possible in the simpler systems with the approach to these classic systems in which some exaggerated feature is studied. This conveys very clearly the decisions that have to be made by the intending research worker.

Garrod's book on *Cellular Development* complements the first book. He concentrates on pattern formation, and includes slime moulds (already discussed by Ashworth), hydra, limb bud formation, insect cuticle, nerve connections and embryonic systems. Simple models suggestively mimic the patterns of development, but the lesson that comes across is that these models are too remote at the moment to shed much light on the molecular basis of development. This basis may be quite subtle and complex, but the challenge is irresistible.

Davies, in *Functions of Biological Membranes,* has made the physicochemical basis of membrane function the central theme, with emphasis on the well studied membrane systems in higher organisms. Gradients (chemical, electrical and osmotic) underlie all significant membrane functions, and have defined characteristics under steady state conditions; these properties certainly need, and here receive, clear exposition. I hope, however, that there will be a companion volume in this series to deal with the nature of the carriers that lend selectivity to transport processes and that couple different transport processes to each other and to a source of energy. Both membrane and transport mutants are the focus of considerable effort in the bacterial systems; Davies could have given clearer pointers to their potential.

Woods has produced a slightly longer reader in *Biochemical Genetics*, selecting and compressing a considerable amount of material in the process. It requires some degree of concentration to follow, for instance when material from prokaryotic and eukaryotic systems is juxtaposed. The book is, however, intended for students who already have some background in genetics. Selection also generates problems—for instance, when the structure of nucleic acids is considered, the melting and annealing properties of DNA are omitted. These properties both illustrate the origin and stability of the double helical structures and underlie the methods for assessment of specific nucleic acid regions or species present in any preparation (including messenger RNA measurements, which give evidence for the transcriptional level of regulation and hybridisation to determine the nature and extent of repetitive genes in eukaryotic cells). The nature of the genetic code is perhaps the most complete section; others on mutants and metabolism and the genetic regulation of metabolism (the systems for lactose and arabinose utilisation and tryptophan production in *Escherichia coli*) are brief. Polar effects of mutations (strongly indicative of operons) are not discussed; this is certain to create some difficulty for the student. The I gene in the *lac* system is not part of the *lac* operon, though both Woods and Ashworth suggest it is.

Written in such a short space, these texts must be compromises, and with only relatively minor points of issue they can be regarded as successful. If the editing can ensure internal cross referencing and consistency, the coverage as the series develops could be extremely useful. There may be many potential authors who already teach suitable material in course form who would be prepared to write at this length, so that it should be possible to maintain the standard already set.

J. F. Collins

Human guinea pigs

Human Guinea-Pigs. By Kenneth Mellanby. Second edition. Pp. 200. (Merlin: London, October 1973.) £2 boards; 80p paper.

The parasitic mite *Sarcoptes scabiei* var. *hominis,* about one sixtieth of an inch long, burrows in the outermost layer of the epidermis. Scabies, the resulting condition of the host, is characterised by intractable itching which, after about three months, becomes severe enough to prevent sleep and, through consequent scratching, to cause sepsis in the skin. Scabies can be an important cause of absence from school and has been a matter of serious concern in the army.

Dr Mellanby describes his experiments on scabies during the Second World War. The mites were believed to be transmitted by bedding and clothing, which was therefore stoved to prevent transmission of the disease. What was not clear was how long the mite could live in the bedding. With forty-seven conscientious objectors, who had volunteered for the work, Dr Mellanby set up a research institute in a Sheffield house to study the mite and the disease. Despite the boredom when without the disease, and the itch and pain when with it, the volunteers loyally and intelligently collaborated to the conclusion of the experiments. It was found that scabies was spread by direct contact, that bedding was not important in transmission, and that two days in a warm airing cupboard sufficed to kill the mite. Thus research costing under £5,000, once and for all, was able to save millions of pounds a year otherwise spent by local authorities on stoving. The volunteers were also subjects for dietetic experiments on calcium balance and, after the scabies work was concluded, remained as a unit under the Medical Research Council for experiments on lack of vitamins A and D. Altogether the work of the human guinea pigs lasted about six years.

This book was first published in 1945 and in this 1973 edition Dr Mellanby has added chapters on the vitamin studies, and on Sir Neil Hamilton Fairley's work with malaria prophylaxis in human volunteers, by which the malaria rate among Australian soldiers in New Guinea was reduced from 740 per thousand in 1943 to an annual rate of twenty-six per thousand in November 1944. Dr Mellanby has also added a chapter in which he extracts from the German concentration camp experiments whatever could be of value, which, as he writes, "make up only a tiny fraction of the atrocities committed ostensibly in the name of medical research".

Dr Mellanby's experience with volunteers lead him to the general conclusions that an institute of human biology, for which subjects could volunteer, could be scientifically valuable; he points out that the Common Cold Unit is at present the only institute at which volunteers regularly serve. Just as he sees possibilities in work with "genuine volunteers, intelligent enough to understand the situation, and always free to terminate their contract", he is cautious whether the word 'volunteer' can ever be properly used for someone imprisoned.

Through the book we learn a little of the author, of the conscientious objectors, and of their understanding of each other, as it developed. Whether the topic is people, the practice of experiments or the author's general conclusions, the writing is always lucid, precise and easy to follow. The manner is modest, without exaggeration of the achievement or unjustifiably wide generalisation from results. This modesty adds force to the author's words when he gives his reasons for preparing the 1973 edition.

One of these is that this work on scabies, of obvious applicability and amply justifiable economically, could never have originated as a Rothschild 'customer-contractor' enterprise. "The work was only done because a very junior research worker thought that something should be done, and then put forward a hare-brained scheme which, miraculously, attracted a modest amount of support, even if . . . only . . . after he had crusaded with missionary zeal".

Finally one may, with Dr Mellanby, note that "the great Viennese dermatologist Hebra discovered almost all the main facts about scabies a hundred years ago, and if proper reliance had been placed on his results, a great deal of human suffering would have been avoided". This lesson is applicable in many fields.

Jeffrey Boss

Words for minds

The Language of Psychoanalysis. By J. Laplanche and J.-B. Pontalis. Pp. xv+510. (International Psychoanalytical Library Series.) (Hogarth Press and Institute of Psycho-Analysis: London, November 1973.) £6.50.

The title of this book suggests that it is an enquiry into the nature of psychoanalytical language, rather in the way

this was recently undertaken by the sociologist Maurice Roche in his *Phenomenology, Language and the Social Sciences.* Such a book would have been welcome especially if written by someone with direct experience of psychoanalytic work. But the title is misleading: the book is a dictionary, and sets out to elucidate, in detail, the accepted meanings of the many concepts used in psychoanalysis, including an account of their evolution.

In a short introduction Daniel Lagache justifies psychoanalytical terminology, maintaining that "ordinary language has no words to evoke mental structures and tendencies that do not exist for common sense." But do mental structures of the kind proposed by Freud exist? Is there really an id? Surely it is the naive acceptance of such an idea that has led Freudian theory into much disrepute? The question as to how to describe "tendencies that do not exist for common sense" is more debatable. Freudian theory seems to have gone astray here too—but it is not alone in so doing, being in the company of traditional psychiatry and psychology and most if not all disciplines which have attempted a "scientific" account of human meaning and behaviour. The more the language departs from that of ordinary common sense the greater it removes itself from psychological reality and in the end we are lost in a mass of jargon and a complexity of tautologies.

Because the authors do not question Freud's basic intellectual stance the book is vulnerable to criticisms of this kind. It lacks the objectivity of Charles Rycroft's delightful but all too short *Critical Dictionary of Psychoanalysis.* But having expressed my dissatisfactions with the book I must confess to an admiration for it. It is clear and scholarly, avoids tautology as far as can be done in the context of the task and gives a tight discussion not only of the usage of terms but some of the relevant problems of psychoanalysis to which they refer.

PETER LOMAS

Cell membrane systems

Comparative Organellography of the Cytoplasm. By A. Frey-Wyssling. (Protoplasmatologia, Vol. III/G.) Pp. vii +106. (Springer-Verlag: Vienna and New York, 1973.) 296 schillings; 42.40 DM; $23.90.

THIS is a short account of the present state of knowledge about the membrane systems, microtubules and microfilaments of plant and animal cells. It is very condensed but because the subject matter is presented from such a definite viewpoint, the ideas and theories are very stimulating. The generalisations do, however, in most cases need more reservations and qualifications than they are given. In this connection it is a pity that in such a short book space is given up to discussions of the Greek origins and other aspects of nomenclature rather than an amplification of conclusions.

Because of its condensed form and the bias that is given to the subject matter the book is rather unsuitable as background reading for elementary students. Most workers in the field will, however, find it very interesting, and although they may not agree with all the ideas the account does give an overall perspective that may well be lost by concentration on one particular aspect of this very important and exciting field of biology.

D. H. NORTHCOTE

Cinderella controllers

Control and Urban Planning. By J. Brian McLoughlin. Pp. 287. (Faber: London, October 1973.) £3.95.

BRIAN McLOUGHLIN draws together two threads in his latest book: his long standing interest in the theory of control, and the results of a research project on development control in planning. He discovered that the development controllers, planning's caseworkers, feel themselves to be the Cinderellas of planning: the whizz kids go into 'strategy' or 'research'. And yet it can be argued, as it is here, that the essence of planning is control, that its main instrument is development control, and so the latter must be connected to the latest research in the field of the former.

The argument is set out at follows. An historical account of the nature and instruments of control in planning, including an effective review of official reports and government publications, is given in chapters 1–5. The implications of the 1968 Act, and structure planning, are taken up in chapter 6. The need for a more explicit treatment of policy making is identified and this is pursued in chapter 7. The theory of control and its application in urban planning, largely from the viewpoint of cybernetics (Ashby, Klir, Valach, and Beer loom large) is presented in chapters 8 and 9, and is connected to the issues for urban planning identified in earlier chapters. Some recommendations are presented in chapter 10.

What emerges from the argument? The early emphasis on development control leads to an exceptionally well balanced approach overall, as this aspect is usually neglected. It also brings to the fore the need for a more explicit treatment of policy. General strategic statements are insufficient for the needs of the development controller. Considerable illumination is cast using the theory of control. Three examples will suffice. First, Beer's concepts of hierarchies in control systems, and associated differences in language between levels, show that there is an as yet unbridged gap between strategy and micro decision levels. Second, Ashby's law of requisite variety helps to determine necessary characteristics of the control system. Third, several levels of resolution are needed, and the two problems cited above will look different at different levels.

All of this will stimulate future research. One of the main roles of the book, however, will be to connect planners who have had a traditional education to these newer ideas, and such exposition has always been McLoughlin's forte. It is a happy accident that the argument is rooted in development control and the issues raised by the demands of structure planning, as the reader is immersed in this before being led gently into control theory. The myth that control theory leads to government by computer is laid bare. The traditional planner will almost be forced to recognise that the new insights will help, and indeed are necessary, for any underpinning value system. The 'new planning' is clearly seen as an aid to the political process and not a substitute for it.

It is possible that a few people will be put off by cybernetic jargon, and it remains an interesting speculation that although this presentation is sufficient, it may not be necessary! There may be other perspectives from which some of the new ideas can be explained to a wider public. It is also possible that the cybernetic viewpoint sometimes forces a view of urban systems as such (as distinct from associated planning processes) which overemphasises, say, homeostatic adjustment, relative to other systems analytical approaches—such as statistical averaging or accounting procedures. But in relation to the main purposes of the book these criticisms are minor. Its arrival is most timely. Urban planners are attempting to be more scientific. The traditional concerns of physical planning are having to be integrated with many other fields of urban government into systems of government. New local authorities take over from the old in April 1974. The author provides an excellent agenda of the issues facing them and many insights towards solutions to problems. The book is well written, easy to read and deserves a wide audience.

A. G. WILSON

science on radio

Burning issues

John Hall

EVEN when we are in the eye of a storm as far as energy resources are concerned, a measure of the seriousness which the BBC attaches to the subject may be gauged from the time slot it allowed Brian J. Ford's assessment of possible solutions, "Filling the Energy Gap", in the series "Where Are You Taking Us?". Radio Four at 10.15 on a Sunday evening is not exactly a prime spot to air a burning issue, especially when it puts a strain on one's loyalties to Monty Python's Flying Circus, which is pitched at a level of concern more appropriate to the tail end of the two-day break. Maybe the non-alarmist conclusion of the programme played a part in its placing; broadly, after examining the evidence, Ford could not forfend remarking that perhaps the Arab induced energy problem was a blessing in disguise. Better to be reminded of the urgent need for energy research by a merely political shut-down than by an actual, *bona fide* exhaustion of the oil, he argued. Which of course is an eminently defensible world view of the situation, though less satisfying as an answer to a Swedish motorist, or a Dutch polythene manufacturer, to whom the distinction between actual and political scarcity must appear academic.

Taking the supra-national view then, the programme pointed up the fact that there is more energy lying around than we know how to use, and once we can shake off an addiction to the destruction by burning of fossil fuels, the sooner we are likely to happen on the technological keys to a hydrogen fusion and fast breeder power strategy.

Evidence for these conclusions was offered by Sir Peter Kent, chairman of the Natural Environment Research Council, and Dr Ian Fell, a fuel technologist working at Newcastle University. The historical basis for the present pretty pass, both men agreed, was that the past cheapness of oil had rendered alternative energy propositions so economically unviable that they had not been adequately researched. A fourfold rise in crude oil prices in one year altered the situation somewhat.

Dr Fell's hopes for the future centred on electrical energy from nuclear fusion; given the technology to contain high temperature plasma, nuclear fusion offered the prospect of all eternity without a worry about energy. Less spectacularly, at least according to present materials costs, the fuel cell might offer a good means of powering traction, given successful research. The Sun, in fact, was held out as the ultimate provider. According to Sir Peter the energy involved in sinking a shaft to tap geothermal sources might require a greater input than could be extracted; and Dr Fell envisaged an expenditure of £1,000 million on a Severn barrage scheme providing 10% of present British electrical requirements. But, free of charge and by courtesy of photosynthesis, solar energy produced an annual equivalent of 100 million tons of coal, largely in the forests of the Third World.

Sir Peter looked for research into ways of taking energy from coal deposits without actually bringing the stuff up in tubs, and Dr Fell asked for work in the pure physics and chemistry of burning, a basic part of energy conversion presently receiving scant attention. Fundamental research apart, simply finding out how to adjust a furnace already in use might effect a saving of 10% to 20%. And on the subject of making the best use of the tools at our disposal, Dr Fell was even hopeful that the current crisis might focus the minds of our politicians wonderfully, so that we should be enabled to emerge, after two or three difficult years, unbowed, if not unbloodied.

One small step

John Gribbin

IT is often said that the best gamekeepers are reformed poachers. On that basis, the choice of John Maddox to present BBC Radio 3's new fortnightly programme "Scientifically Speaking" seemed ideal, as anyone who was a regular reader of *Nature* a year or so ago will appreciate. But unfortunately the possibilities raised by this choice were left unfulfilled, in the first programme at least, and Maddox was heard to be cautiously feeling his way, making one small step forward rather than taking a giant leap into the new lands of broadcasting.

The start of the programme promised well, with Maddox putting over his own view and asking why science should not be popular and whether it is sensible to starve research of funds, and commenting on the passive way the research councils toe the line of government restrictions. Maddox riding these hobby horses is worth listening to and makes a lot of sense. But when it came to putting over recent advances in science, rather than commenting on the politics of science, one of the questions was answered: science lacks popularity, to some extent at least, because it is put over in such a dull way by programmes such as this.

Science is fun, as Maddox is fond of saying. But you would never guess it from this programme. I can find no fault with the choice of subjects (spreading of the Red Sea, Professor R. A. Lyttleton's view on comets, hepatitis B and the possibility of determining the sex of children in advance). Even these exciting subjects, however, came over in the usual interviewer/interviewee format, full of indigestible facts. Maddox is not the best interviewer in the world, and the indigestibility of the facts was compounded by his own difficulties, especially with Lyttleton, who allowed the questioner little use of the microphone.

Comet Kohoutek passed some 7 to 8 million miles from the Sun, said Maddox; actually no, more like 13 million miles, corrected Lyttleton. But, he added, there was one comet that came within a hundred thousand kilometres. Halley's Comet was mentioned—due in 1996 (Maddox) or 1986 (Lyttleton) depending on how carefully you were listening. And summing up this discussion Maddox told us that a space probe sent to the tail of a comet would resolve the dust cloud/icy nucleus argument; wrong again (although this time Lyttleton was not present to correct the error); the probe should, of course, be sent to a comet's nucleus.

The story of choosing a baby's sex offered plenty of scope for fun, none of which was taken up. More interest would have been aroused, surely, by reporting the folk-lore tale that pilots of jet fighters tend chiefly to have daughters, because of the effect of high *g* on sperm, and asking the experts to account for that in terms of the recent Schering work.

These may be the subjects about which scientists are excited, but you would never guess so from this kind of interview. Maddox is at his best as a commentator and all that this programme needs to become really worthwhile is to use him in that role while unloading some of the interviewing donkey work on to someone more adept at the task. If the powers that be at the BBC could then be persuaded to ensure that it did not clash with the only regular science programme on television ("Horizon") those of us who have been lobbying for more science programmes would really have something to shout about.

obituary

Sir Robert Waston-Watt

Sir Robert Watson-Watt, pioneer of radar, died on December 5, 1973. He was 81 years old and had been in poor health for some time.

He obtained his early education in Brechin, his birthplace, and proceeded with a bursary to University of St Andrews where, at University College, Dundee, he studied electrical engineering. After graduation he was appointed Assistant to the Professor of Natural Philosophy at Dundee, a post which he occupied until 1915 when he became an assistant at the Branch Meteorological Office at the Royal Aircraft Factory, Farnborough.

It was during his service at Farnborough that he took up the study of thunderstorms by means of the radio waves, or atmospherics, emitted by them. After the end of hostilities, work on atmospherics and their relation to meteorological phenomena became the main investigation of the station of which he had been made Meteorologist-in-Charge in 1917. He continued in charge of the station after its transfer to the Department of Scientific and Industrial Research in 1921 and its removal to Slough in 1924. In 1927 he became Superintendent of the Radio Research Station set up to absorb the atmospherics work and other radio research of the Department of Scientific and Industrial Research in progress at Slough.

In his early studies of atmospherics one of the main problems was the location of their sources. Although some success on streams of atmospherics was obtained using Bellini-Tosi direction finders, it was Watson-Watt's invention of the instantaneous visual cathode-ray direction finder (CRDF) which enabled the source of a single atmospheric to be found and greatly facilitated the whole investigation.

During the Second World War Watson-Watt's CRDF apparatus was operated by the Meteorological Office as a means of observing the positions of thunderstorms around the British Isles in areas from which no synoptic reports were then received. Similar equipment is still regularly used by the Meteorological Office to help in the production of weather forecasts.

The ability of the CRDF to work on short duration signals found an important application during the Battle of the Atlantic during which ship- and shore-based apparatus was used to locate U-boats from bearings obtained on their brief radio transmissions.

Watson-Watt will, however, be best remembered for his pioneering work in radar. This began early in 1935 when he was approached informally by the Air Ministry for an independent appreciation of the possibility of generating a radio 'death-ray' for use against enemy aircraft. While rejecting the feasibility of such a ray, he reported that the detection and location of aircraft at useful ranges was possible and subsequently submitted a memorandum giving his proposals on how this could be done to Sir Henry Tizard's Committee for the Scientific Survey of Air Defence. After a successful demonstration of the underlying principles he was requested to begin development work along the lines indicated in the memorandom. This he did at Orfordness where the work prospered rapidly and was soon moved to Bawdsey, near Felixstowe.

Appointed Superintendent of Bawdsey Research Station in August 1936 he and his team devoted themselves energetically to those forms of radar, both ground-based and airborne, which were to play such a vital part in the Second World War.

After the successful initiation of radar, Watson-Watt, while continuing to make important technical contributions, found his main activity in cogent advocacy of radar in Whitehall and in stimulating the bureaucratic machine into unwonted activity to provide the material requirements of the research worker and of the coastal stations. These activities led, in 1938, to his appointment as Director of Communications Development, Air Ministry, but soon after the outbreak of war, his talents found more congenial use as Scientific Advisor on Telecommunications at both the Air Ministry and Ministry of Aircraft Production, and as Vice Chairman of the Radio Board of the War Cabinet.

After the war he retired from Government service to conduct a consultancy business, mainly in North America, which he set up with wartime colleagues.

Amongst the many honours he received were C. B. and Knight Bachelor; he was a Fellow and Hughes Medallist of the Royal Society and held honorary doctorates of St Andrews, Toronto and Laval Universities. He was a Past President of the Royal Meteorological Society and of the Institute of Navigation.

A man of great gifts and wide interests he was a genial, courteous and well-informed companion.

Sir Ronald Holroyd

Sir Ronald Holroyd, FRS, who died on September 29, was an outstanding industrial scientist. His genius lay not in success as an individual worker, but in a capacity to organise large-scale research projects that were the foundation of important new processes. His field was coal and petroleum chemistry, and he was responsible for developments which were of major national importance during and immediately after the second world war.

Holroyd was born in Yorkshire on April 26, 1904. After going to Holgate Grammar School, Barnsley, he read chemistry at Sheffield University (1921–25). After a short period with the Board of Trade, he joined ICI in 1928. Originally with Brunner, Mond at Winnington, he transferred to Billingham in 1932. In 1947 he was appointed research director of Billingham Division, and five years later joined the ICI Main Board in London, quickly becoming research director (1935). In 1957 he was appointed a Deputy Chairman, and held this position until he retired in 1967.

At Billingham, Holroyd was particularly concerned with the production of liquid hydrocarbons, including petrol, by the hydrogenation of coal and creosote, and a plant was put into operation in 1935. The outbreak of war in 1939 gave new urgency to this process, especially for the supply of aviation fuel, because of the lack of petroleum-refining capacity in Britain. In 1941 a consortium of ICI, Shell, and Trinidad Leaseholds set up a gas-oil hydrogenation plant at Heysham, and in the latter years of the war some half million tons of aviation base spirit was produced in Britain annually. Among technical problems overcome was that of increasing the octane rating, partly by increasing the aromatic content and partly by additives such as tertiary butyl benzene (Victane) and monomethyl aniline. This was of crucial importance in enabling Spitfires to catch German V 1 rockets. Apart from their intrinsic importance, these developments resulted in an accumulation of knowledge and experience of hydrocarbon chemistry, which was invaluable in the post-war chemical revolution when petroleum almost wholly superceded coal-tar as a raw material.

An off-shoot of this work was the development of analytical techniques for hydrocarbons, especially infra-red and ultraviolet spectroscopy. Analysis of fuel from shot-down enemy aircraft threw much light on the German oil supply situation. In the closing stages of the war, Holroyd led several teams which went to Germany to investigate oil and aviation fuel developments there.

Holroyd's contributions were widely recognised. He was elected Fellow of the Royal Society in 1960, and was knighted in 1963. He was an honorary graduate of Oxford, Sheffield, Hull, and Trinity College Dublin. In 1958 he was awarded the Castner Medal of the Society of Chemical Industry, of which he was President 1965–7.

matters arising

Mesozoic rocks from the Labrador sea

BECAUSE of the great interest, both academic and commercial, in the samples reported by Johnson *et al.*[1], additional stratigraphic analyses were conducted in Pau, France, by Aquitaine Company of Canada (Table 1). These analyses were concentrated on the nannofossils; especially the coccoliths (G. Chennaux and S. Mulle, unpublished). As noted by Chennaux and Mulle (Tabe 1) the samples show a heterogeneity of ages ranging from Mesozoic to Recent. The Mesozoic nannofossils are in a poor state of preservation in contrast to well preserved Tertiary nannofossils, which supports the younger age. The presence of Mesozoic spores and pollens[1] are therefore assumed to be reworked organic debris deposited in the prograding Tertiary sequences.

A Tertiary age for these samples would be in agreement with seismic velocities from the outer Greenland shelf (N. J. McMillan, personal communication). An Oligocene date plots along a more normal subsidence rate of 30–40 m per m.y. (ref. 1, Fig. 2).

These revised ages for Greenland samples suggest that the Mesozoic ages reported from the Labrador continental margin[2] may need revision too.

We thank J. Aubert, F. Deres, C. Boulovard, G. Peniguel, and C. Poumot all of Centre de Recherches de Societe Nationale des Petroles d'Aquitaine, Pau,

TABLE 1 Revised data on South-west Greenland dredge samples

		Age of associations		Age proposed by Johnson *et al.*[1]	
Sample No.	Position	Nannofossils	Palynoplanktology	Palynoplanktology	Revised age
32	60°04.8′N 46°44.0′W	Oligocene (and remains of Lower Cretaceous)	Lower Palaeozoic Triassic Cretaceous to Jurassic	Upper Cretaceous (and Jurassic remains)	Oligocene
37	60°04.6′N 47°10.5′W	Oligocene to Lower Miocene	Undetermined Probably Recent	Upper Cretaceous (Albian or younger) (and Jurassic remains)	Oligocene
43A	63°34.3′N 52°57.0′W	Oligocene	Lower Cretaceous	Upper Cretaceous	Oligocene
43B	63°34.3′N 52°57.0′W	Probably Eocene	Cretaceous? Triassic? Carboniferous?	Upper Cretaceous (Turonian—Senonian Lower) (and Jurassic and Upper Carboniferous relics)	Eocene
56	61°35.5′N 50°37.1′W	Eocene–Oligocene (and Jurassic remains)	Lower Cretaceous Recent? Triassic	Upper Cretaceous (and Jurassic and Upper Carboniferous relics)	Eocene to Oligocene
59A	61°53.0′N 50°45.0′W	Middle Eocene to Oligocene	Lifeless	Upper Cenomanian to Lower Turonian	Oligocene
59B	61°53.0′N 50°45.0′W	Lifeless	Lower Cretaceous Triassic	Upper Cenomanian to Lower Turonian	
62A	62°35.0′N 51°35.0′W	Lifeless	Triassic to Cretaceous	Pliocene to Recent (and Cretaceous, Jurassic and Palaeozoic)	Pleistocene? to Recent
62B	62°35.0′N 51°35.0′W	Probably Recent	Recent? Lower Cretaceous Triassic	Turonian	Pleistocene? to Recent
65	63°07.5′N 52°17.8′W	Lower Palaeocene	Lower Cretaceous	Jurassic lower (Bajocian or Pliensbachian) and undetermined	Lower Palaeocene
69	64°25.0′N 52°53.0′W	Tertiary (and remains of Cretaceous)	Undetermined	Palaeocene (and Jurassic remains)	Palaeocene

France who conducted the biostratigrapic studies, and J. McMillan of Aquitaine Company of Canada who reviewed the manuscript.

G. L. JOHNSON

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Washington DC 20373

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Oester Voldgade 5-7
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[1] Johnson, G. L., Campsie, J., Rasmussen, M., and Dittmer, F., *Nature phys. Sci.*, **236**, 86 (1972).
[2] McMillan, N. J., *Geol. Surv. Can. Pap.*, 71-73, 451 (1973).

Announcements

International Meetings

February 28, **The Role of Satellites in Sensing the Global Atmosphere and their Application in Weather Forecasting** (General Secretary of the Remote Sensing Society, Dr W. G. Collins, Reader in Remote Sensing, Remote Sensing Unit, University of Aston, Gosta Green, Birmingham, B47ET)

February 28–March 1, **7th International TNO Conference** (The Secretariat, c/o Holland Organising Centre, 16 Lange Voorhout, The Hague, Netherlands)

Errata

In the News and Views article, "From Cyclic AMP to Cyclic GMP" (*Nature*, **246**, 186; 1973) reference was made to volume 3 of *Recent Advances in Cyclic Nucleotide Research* by Goldberg *et al.* This volume is actually entitled *Advances in Cyclic Nucleotide Research* and will be published by Raven Press.

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In the review of the book *The Estimation of Animal Abundance and Related Parameters* by G. A. F. Seber (*Nature*, **246**, 536; 1973) the number of pages should have been given as 506, not 56.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

British Antarctic Survey. Scientific Reports, No. 68: Vegetation of the South Orkney Islands, with particular reference to Signy Island. By Dr. R. I. Lewis Smith. Pp. 124+6 plates. (London: British Antarctic Survey, Natural Environment Research Council, 1972.) £3.75 net. [110

Social Science Research Council. Annual Report 1972/1973. Pp. 76. (London: HMSO, 1973.) 47p net. [410

Bulletin of the British Museum (Natural History). Entomology. Vol. 28, No. 1: The Genus *Etiella* Zeller (Lepidoptera: Pyralidae)—a Zoogeographic and Taxonomic Study. By P. E. S. Whalley. Pp. 1–21+15 plates. £2.60. Vol. 28, No. 4: A Catalogue of the Family-Group and Genus-Group Names of the Gelechiidae, Holcopogonidae, Lecithoceridae and Symmocidae (Lepidoptera). By K. Sattler. Pp. 153–282. £4.70. Geology. Vol. 23, No. 4: Palaeozoic Coral Faunas from Venezuela, II. Devonian and Carboniferous Corals from the Sierra de Perija. By C. T. Scrutton. Pp. 221–281+10 plates. £4.35. Vol. 23, No. 5: *Prodeinotherium* from Gebel Zelten, Libya. By J. M. Harris. Pp. 283–348+5 plates. £2.85. Vol. 23, No. 6: Cirripedes from the Upper Cretaceous of Alabama and Mississippi, Eastern Gulf Region, U.S.A. I. Palaeontology. By J. S. H. Collins. II. Geology. By F. F. Mellen. Pp. 381–388. £2.25. Vol. 23, No. 7: Fenestrate Bryozoa from the Visean of County Fermanagh, Ireland. By R. Tavener-Smith. Pp. 389–493+26 plates. £7. Historical Series. Vol. 4, No. 4: The Entomological Publications of Pierre Millière (1811–1887). By K. Sattler and W. G. Tremewan. Pp. 221–280+3 plates. £2.70. Mineralogy. Vol. 2, No. 6: The Pseudoleucite Borolanites and Associated Rocks of the South-Eastern Tract of the Borralan Complex, Scotland. By A. R. Woolley. Pp. 285–333+6 plates. £2.95. Vol. 2, No. 7: Rocks from Antarctica—the *Discovery* Collection in the British Museum (Natural History). By D. R. C. Kempe. Pp. 335–376+7 plates. £2.45. Zoology. Vol. 25, No. 1: On the Cichlid Fishes of the Genus *Pelmatochromis* with Proposal of a New Genus for *P Congicus*; on the Relationship Between *Pelmatochromis* and *Tilapia* and the Recognition of *Sarotherodon* as a Distinct Genus; and A New Species of Cichlid Fishes of Rivers Quanza and Bengo, Angola, with a List of the Known Cichlidae of these Rivers and a Note on *Pseudocrenilarbus natalensis* Fowler. By E. Trewavas. Pp. 1–37+1 plate. £1.65. Vol. 25, No. 3: Freeliving Marine Nematodes from the Indian Ocean. By R. M. Warwick. Pp. 85–117. £1.50. Vol. 25, No. 4: Biology and Fine Structure of *Euglypha rotunda* (Testacea: Protozoa). By R. H. Hedley and C. G. Ogden. Pp. 119–137+7 plates. £1.60. Vol. 25, No. 5: A Revision of the *Haplochromis* and Related Species (Pisces: Cichlidae) from Lake George, Uganda. By P. H. Greenwood. Pp. 139–242. £4.55. Vol. 25, No. 6: Preliminary Notes on the Ontogeny of the Frontal Body Wall in the Adeonidae and Adeonellidae (Bryozoa, Cheilostomata). By P. L. Cook. Pp. 243–263+3 plates. £1.45. Vol. 25, No. 7: Some New Taxa of Recent Stalked Crinoidea. By A. M. Clark. Pp. 265–288+2 plates. £1.50. (London: British Museum (Natural History), 1973.) [1010

Proceedings of the Royal Irish Academy. Vol. 73, Section B, No. 12: Pleistocene Glacial, Glaciomarine and Associated Deposits of Mell and Tullyallen Townlands, Near Drogheda, Eastern Ireland. By E. A. Colhoun and A. M. McCabe. Pp. 165–2–6+plates 11–14. (Dublin: Royal Irish Academy, 1973.) 64p. [1610

Memoirs of the Royal Astronomical Society, Vol. 77, Part 5: Radial Velocities of Southern B Stars Determined at the Radcliffe Observatory—VII. By A. D. Thackeray, S. B. Tritton and E. N. Walker. Pp. 203–222. (Oxford and London: Blackwell Scientific Publications, 1973. Published for the Royal Astronomical Society.) [1710

People, Vol. 1, No. 1, October 1973. Published quarterly. Pp. 1–48. (London: International Planned Parenthood Federation, 18/20 Lower Regent Street, SW1, 1973.) [1710

Other Countries

Smithsonian Contributions to Zoology, No. 141: Morphology of *Cypretta kawatai* Sohn and Kornicker, 1972 (Crustacea, Ostracoda), with a Discussion of the Genus. By I. G. Sohn and Louis S. Kornicker. Pp. 28. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) [illegible]0 cents. [110

United States Department of the Interior: Geological Survey. Professional Paper 433-M: Relations Among Radionuclide Content and Physical, Chemical, and Mineral Characteristics of Columbia River Sediments. By J. L. Glenn. With a Section on Sand and Gravel Mineralogy by R. O. Van Atta. Pp. iii+52. 85 cents. Professional Paper 751-B: Design and Operation of the Artificial-Recharge Plant at Bay Park, New York. By Ellis Koch, Anthony A. Giaimo and Dennis J. Sulam. Pp. iii+14+plate 1. 95 cents. Professional Paper 754-A: Glacial and Postglacial Geologic History of Isle Royale National Park, Michigan. By N. King Huber. Pp. iii+15+plate 1. $1. Professional Paper 809-A: Recognition of Natural Brine by Electrical Soundings Near the Salt Fork of the Brazos River, Kent and Stonewall Counties, Texas. By Adel A. R. Zohdy and Dallas B. Jackson. Pp. iii+14. 40 cents. (Washington, DC: Government Printing Office, 1973.) [210

United States Department of the Interior: Geological Survey. Water-Supply Paper 2028: A National Study of the Streamflow Data-Collection Program. By Manuel A. Benson and Rolland W. Carter. Pp. iv+44. 45 cents. Bulletin 1309: Chemical Analysis of Thermal Waters in Yellowstone Park, Wyoming, 1960–65. By J. J. Rowe, R. O. Fournier and G. W. Morey. Pp. iii+31. 35 cents. Professional Paper 790: Stratigraphy, Morphology, and Paleoecology of a Fossil Peccary Herd from Western Kentucky. By Warren I. Finch, Frank C. Whitmore, Jr., and John D. Sims. Pp. iii+25. 70 cents. Professional Paper 712-B: Geohydrology of the Eastern Part of Pahute Mesa, Nevada Test Site, Nye County, Nevada. By Richard K. Blankennagel and J. E. Weir, Jr. Pp. iv+35+plates 1–3. $1.75. Professional Paper 685: Fossils from the Ordovician Bioherm at Meiklejohn Peak, Nevada. By Reuben James Ross, Jr. Pp. iv+47+plates 1–18. $1.25. Professional Paper 663-B; Structural Geology of the Sun River Canyon and Adjacent Areas, Northwestern Montana. By Melville R. Mudge. Pp. vi+52+plates 1–5. (Washington, DC; Government Printing Office, 1972 and 1973.) [310

Smithsonian Contributions to Zoology, No. 145: The Frog Genus *Atelopus* in Ecuador (Anura: Bufonidae). By James A. Petera. Pp. 49. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) 60 cents. [310

The Flaw in the Michelson-Morley Experiment. By V. Mabry. Pp. 14. (Beaumont, Texas: Virden Mabry, 2310 Angelina Street, 1973.) [810

Australia. Commonwealth Scientific and Industrial Research Organization. Protein Chemistry—Review and Abstracts, 1972. Pp. 64. (Parkville, Victoria: Division of Protein Chemistry, CSIRO, 1973.) [1010

United States Department of the Interior: Geological Survey. Professional Paper 313-F: Geology and Phosphate Deposits of the Permian Rocks in Central Western Montana. By Roger W. Swanson. Pp. iv+779–833+plates 30–34. (Washington, DC: Government Printing Office, 1973.) [1010

Stockholm International Peace Research Institute. SIPRI Research Report No. 10: The Meaning and Measurement of Military Expenditure. Pp. 39. (Stockholm: Stockholm International Peace Research Institute, 1973.) [1110

United States Department of the Interior: Geological Survey. Professional Paper 726-D: Geophysical Studies in the Yuma Area, Arizona and California. By R. E. Mattick, F. H. Olmsted and A. A. R. Zohdy. Pp. iv+36+plates 1–5. (Washington, DC: Government Printing Office, 1973.) $1.75. [1110

OECD, Paris. Uranium: Resources, Production and Demand. (A Joint Report by the OECD Nuclear Energy Agency and the International Atomic Energy Agency. Pp. 140. (Paris: OECD; London: HMSO, 1973.) 20 francs; £1.76. [1210

Fisheries Research Board of Canada. Bulletin 181: Population Dynamics of the Ringed Seal in the Canadian Eastern Arctic. By Thomas G. Smith. Pp. 55. $2.50. (Ottawa: Information Canada, 1973.) [1210

United States Department of the Interior: Geological Survey. Bulletin 1372-J: Stratigraphic Terminology of the Dakota Sandstone and Mancis Shale, West-Central New Mexico. By E. R. Landis, C. H. Dane and W. A. Cobban. Pp. iii+44. (Washington, DC: Government Printing Office, 1973.) 30 cents. [1510

Unesco. International Classification and Mapping of Vegetation. (Ecology and Conservation, 6.) Pp. 91. (Paris: Unesco; London: HMSO, 1973.) 48 francs; £4; $12. [1510

Geology, Vol. 1, No. 1, September 1973. Published monthly. Pp. 1–48. Subscriptions: $9 per year for the United States, Canada and Mexico, and $11 elsewhere. Single copies 75 cents. (Boulder, Colorado: The Geological Society of America, Inc., 3300 Penrose Place, 1973.) [1510

World Health Organisation. Technical Report Series, No. 531: The Use of Viruses for the Control of Insect Pests and Disease Vectors—Report of a Joint FAO/WHO Meeting on Insect Viruses. Pp. 48. (Geneva: WHO; London: HMSO, 1973.) 4 Sw. francs; 50p; $1.20. [1610

CERN—European Organisation for Nuclear Research. CERN 73-11: High-Accuracy Measurements of the Centre of Gravity of Avalanches in Proportional Chambers. By G. Charpak, A. Jeavons, F. Sauli and R. Stubbs. Pp. 4. (Geneva: CERN, 1973.) [1610

Smithsonian Contributions to Zoology, No. 120: A Systematic Monograph of New World Ethmiid Moths (Lepidoptera: Gelechioidea). By Jerry A. Powell. Pp. iv+302 (22 plates). Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) $3.50. [1710

United States Department of the Interior: Geological Survey. Professional Paper 838: Geologic and Seismologic Aspects of the Managua, Nicaragua, Earthquakes of December 23, 1972. By R. D. Brown, Jr., P. L. Ward and George Plafker. Pp. vi+34+plate 1. (Washington, DC: Government Printing Office, 1973.) [1710

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DEPARTMENT OF CLINICAL CHEMISTRY

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Applications are invited for a post of Lecturer in the Department of Clinical Chemistry, which is situated in the Royal Infirmary of Edinburgh. The successful candidate will be expected to take up the post as soon as possible after August 1, 1974. Candidates may be medical graduates or science graduates holding Honours degree in Biochemistry, Chemistry, or one of the related sciences. Previous experience of work in a hospital laboratory is not essential, although it would be an advantage. Salary range £2,562-£4,866 with FSSU provision. The South-Eastern Regional Hospital Board (Scotland), or its successor, will be prepared to grant the appropriate honorary status to the successful candidate. Applicants may visit the Department by prior arrangement (Telephone 031-229 2477, extension 2319).

Further particulars of the appointment may be obtained from The Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL, with whom applications (three copies), giving the names of two referees, should be lodged by March 8, 1974. Please quote reference number 1003.

(567)

SHEFFIELD POLYTECHNIC

DEPARTMENT OF CHEMISTRY AND BIOLOGY

SENIOR LECTURER/LECTURER (GRADE II) IN CHEMISTRY

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To teach a wide range of the biological sciences, but particularly in the fields of animal physiology and comparative physiology.

For appointment at the Senior Lecturer level a candidate must also have considerable experience of teaching biological sciences at undergraduate level.

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Salary scales: Senior Lecturer, £3,291-£3,655 (bar)-£3,928; Lecturer II, £2,515-£3,243.

Application forms and further details obtainable from the Staffing Officer, Sheffield Polytechnic, Halfords House, Fitzalan Square, Sheffield S1 2BB, to whom completed forms should be returned within 14 days. (543)

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The post is superannuable. Salary will be within the range £1,809 to £2,820. An essential user car allowance is provided.

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CLYDE RIVER PURIFICATION BOARD

(512)

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Clear and accurate expression in the written word is essential with English as the mother tongue.

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Applications to:

Mr. D. S. Sweetman,
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Paramount House, 78 Uxbridge Road, Ealing, London W5 5SS.

(575)

In association with its Institute of Molecular Genetics in Berlin-Dahlem the

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Two of these positions will be available, one starting on or after April 1974 and the second on or after January 1975.

Applicants should already have done some recognised research work. Appointments will be limited up to 5 years. Salaries will fall within the range AH 3 of the Niedersächsisches Besoldungsgesetz (about DM 45.000, per annum).

Applications should be submitted before April 15, 1974, to

Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.—Generalverwaltung—III a 1–8000 München 1, Postfach 647, Germany.

(532)

CSIRO

AUSTRALIA

DIVISION OF ANIMAL PHYSIOLOGY, PROSPECT, N.S.W.

RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD: **REPRODUCTIVE PHYSIOLOGY**

GENERAL: The Ian Clunies Ross Animal Research Laboratory is the Headquarters of the Division of Animal Physiology and is situated at Prospect, 20 miles west of Sydney, N.S.W. The Laboratory has a total staff of 150, including 55 professional scientists. Past and current research of the Laboratory includes work on endocrinology, nutrition, reproduction and lipid metabolism.

The Laboratory has a broadly-based programme of research on problems of reproduction and production in cattle and sheep. A physiologist or biochemist is required to collaborate with physiologists and endocrinologists in a study of the physiology of the hypothalamic releasing factors and their application to field problems.

DUTIES: To conduct research into the physiology of hypothalamic releasing hormones, and in the first instance, their use in the induction of ovulation during lactational or seasonal anoestrus. It is envisaged that some work on the isolation of releasing hormones will be required.

QUALIFICATIONS: A Ph.D. degree or equivalent qualifications together with demonstrable research ability. Experience in the endocrinology of hypothalamic and pituitary hormones and with immunoassay methods would be an advantage.

SALARY: Appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: $A9,380–$A14,290 p.a. An outstanding applicant may be offered appointment at a higher level.

TENURE: Either an indefinite or a fixed term appointment may be negotiated. An indefinite appointment carries Australian Government Superannuation benefits.

Applications, stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number: 245/575, should reach:

The Personnel Officer, Australian Scientific Liaison Office,
64–78, Kingsway, LONDON, WC2B 6BD, by the 1st March, 1974.

Applications in U.S.A. and Canada should be sent to:
The Counsellor (Scientific), Embassy of Australia,
1601 Massachusetts Avenue, N.W., WASHINGTON, D.C. 20036,
U.S.A.

(487)

COLÁISTE NA hOLLSCOILE GAILLIMH

(University College Galway)

TECHNICIAN

Applications are invited for the position of TECHNICIAN in the Department of Pathology.

Salary within the region of £1,665 by £72 to £1,953.

Minimum qualifications: Intermediate Certificate of Institute of Medical Laboratory Technology, Intermediate Certificate of City and Guilds of London, Intermediate Technological Certificate of Department of Education or equivalent and O.N.C. Certificate or equivalent.

Applications, stating age, experience, qualifications and the names of two referees, should be sent to Professor of Pathology, Regional Hospital, Galway.

(551)

UNIVERSITY OF LANCASTER

DEPARTMENT OF ENVIRONMENTAL SCIENCES

Applications are invited for TWO LECTURESHIPS in Environmental Sciences. The Department is seeking to strengthen the existing subject areas of geology and hydrology, particularly within the fields of engineering geology and of sedimentary geochemistry. Persons appointed to both posts would be expected to contribute to the Departmental teaching and research programmes in these subject areas. In addition, a contribution to the geomorphological teaching would be a valuable asset. The Department wishes to give more emphasis to applied research topics and it would be expected that the new appointments help expand such activities.

Further particulars may be obtained (quoting reference L 800/D) from the Establishment Officer, University House, Bailrigg, Lancaster LA1 4YW, to whom applications (five copies), naming three referees, should be sent not later than March 15, 1974. (492)

KENYATTA UNIVERSITY COLLEGE

PROFESSOR IN BOTANY: Applicants should be holders of a Ph.D. degree and must have extensive and proven experience in teaching and research at University level. Ability and enthusiasm to plan, initiate and direct research as well as ability to plan and execute new teaching programmes for both graduates and undergraduates are essential qualifications.

SENIOR LECTURESHIP/LECTURESHIPS: Applicants should be holders of a Ph.D. degree in the relevant fields. Applicants for Senior Lectureships should have considerable experience in teaching and research at University level whereas it will be an advantage for applicants of Lectureships.

SENIOR LECTURESHIPS: Physics, Chemistry, Mathematics, Botany and Zoology.

LECTURESHIPS: Physics, Chemistry, Mathematics, Botany (Genetics or Terrestrial Ecology), Zoology (Parasitology or Comparative Anatomy and History or Developmental Biology or Microbiology).

Kenyatta University College is basically a teacher training institution and therefore experience in teacher training at University level, will be an added advantage to applicants for all the posts.

Terms of service include subsidised housing, membership of non-contributory medical scheme and some College contribution towards F.S.S.U. or payment of gratuity at expiry of contract. Expatriate appointments are on tours, each of two years, renewable and includes passages for up to 5 adults on appointment, between tours and on expiry of contracts.

Salary scales: Professor: K£3,600 by K£156 to K£4,380 per annum. Senior Lecturer: K£2,256 by K£108 to K£2,904 by K£132 to K£3,036 per annum. Lecturer: K£1,500 by K£84 to K£2,256 by K£108 to K£2,580 per annum.

Applicants should give names and addresses of three academic referees and at the same time ask them to send their references direct to the Registrar without delay.

Written applications (six copies) giving full details of curriculum vitae including fields of specialisation, age, present post, marital status, etc., should be addressed to: the Registrar, Kenyatta University College, P.O. Box 43844, Nairobi, to reach him not later than February 28, 1974.

All the above posts are for the 1974/75 academic year which begins on July 1, 1974. (482)

DEPARTMENT OF GERIATRICS

KING'S COLLEGE HOSPITAL, SE5

We seek a RESEARCH BIOCHEMIST who would be willing to undertake an investigation into lipid metabolism. Experience in physiology essential. Those interested should contact Dr Brian Livesley in the first instance (Tel: 01-693 3377, ext 3422).

Application forms obtainable from the Personnel Office, King's College Hospital, Denmark Hill, SE5. Tel: 01-274 6222 ext 2728 (Mrs Child) should be completed and returned by two weeks after the appearance of this advertisement. (516)

CELLULAR AND MOLECULAR BIOLOGY

A senior position at the rank of FULL OR ASSOCIATE PROFESSOR is available in the Department of Biology, York University, Toronto, tenable July 1, 1974. Preferred areas of research interest are: genetics and biochemistry of development, cellular and molecular genetics, neuro-biology. Exceptional junior candidates may also be considered. Salary negotiable and commensurate with rank.

Send curriculum vitae, reprints and names of three referees to Professor J. D. Friesen, Department of Biology, York University, 4700 Keele Street, Downsview, Ontario M3J 1P3, Canada. (478)

Veterinary Research up to £6460

Superintendent

Allington Farm, Porton Down

Allington Farm, Porton Down, Salisbury is responsible for supplying experimental animals to the Microbiological Research Establishment and the Chemical Defence Establishment. The Farm also provides large farm animals, and undertakes mixed farming.

Apart from ensuring that animals meet the requirements of each of the Establishments, the Superintendent will be responsible for a continuous programme of research designed to improve the quality and strain of experimental animals.

Candidates should have experience in veterinary pathology or surgery and in breeding and producing experimental animals. They must be able to instigate and supervise research and have proven management ability. A good honours degree in an appropriate subject is desirable.

Starting salary will be within the Senior Principal Scientific Officer scale £5550-£6460. Non-contributory pension scheme. Promotion prospects.

For full details and an application form (to be returned by 22 February 1974) please write to Civil Service Commission, Alencon Link, Basingstoke, Hants. RG21 1JB, or telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24-hour answering service). Please quote S/8519. (528)

MINISTRY OF DEFENCE

GOVERNMENT OF BRUNEI

AGRONOMIST

Required by the Department of Agriculture to take charge of the Agronomy Section and be responsible for an extensive programme of investigations into swamp and rainfed rice, upland crops, tree crops, pastures and horticultural crops. Male candidates only, between 30 and 48 years of age MUST have a degree in Agronomy, Agricultural Science or equivalent and at least 10 years experience of tropical Agronomy (especially the Humid Tropics).

Commencing salary including inducement allowance will be in the range £2,710 to £4,840 a year according to qualifications and experience. A substantial Gratuity is payable on completion of engagement, which is on agreement for 3 years initially.

Other benefits include free passages, liberal leave on full salary, outfit allowance, education allowances and Government Quarters at moderate rental. **There is no personal income tax in Brunei at present.**

For further particulars you should apply, giving brief details of experience, to:

crown agents

M Division, 4 Millbank, London SW1P 3JD, quoting reference number M3F/731108/NF.

(547)

BIOLOGICAL OCEANOGRAPHER MARINE BIOLOGIST

Brookhaven National Laboratory, located on Long Island about 65 miles east of New York City, seeks marine biologist with Ph.D. or equivalent and outstanding record of research to initiate a program in biological oceanography in the north-eastern coastal zone. Successful candidate will be expected to play a major role in making further appointments in this area.

Send curriculum vitae, list of publications, reprints of two recent papers, and three professional references to: Dr John M. Olsen, Search Committee Chairman, Biology Department,

Brookhaven National Laboratory,
Associated Universities Inc.,
Upton, New York 11973

An Equal Opportunity Employer M/F

(X325)

UNIVERSITY
OF DUBLIN
Trinity College

JUNIOR LECTURER IN GENETICS

Applications are invited for the above post.

Salary scale: £1,969 by £126 to £2,473.

Marriage and children's allowances are paid and there is a non-contributory F.S.S.U.-type Pension Scheme.

Further particulars and application forms may be obtained from:

The Staff Secretary,
West Theatre,
Trinity College,
Dublin 2,

who will receive completed applications until Friday, March 1, 1974. (553)

THE ANIMAL VIRUS RESEARCH INSTITUTE

PIRBRIGHT, WOKING, SURREY

VACCINE RESEARCH DEPARTMENT

Applications are invited for a post in the above Department. The successful candidate will work in a small team on the selection and development of new foot-and-mouth disease vaccine strains. The work will include assessment of new strains on the laboratory scale for growth properties and immunogenicity. Experience in virus work is essential, and interests in viral immunology and strain selection procedures would be an advantage. Applicants should have a good Honours degree in microbiology or allied subject and some years' experience as a postgraduate research worker. Appointment in S.S.O. grade (£2,615-£3,640), depending on age and qualifications. Superannuation under F.S.S.U., with allowance to offset cost of contributions. House available for suitable married applicant.

Further particulars and application forms from Secretary. (432)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

DENMARK HILL, LONDON SE5 8RX

DEPARTMENT OF BIOCHEMICAL PHARMACOLOGY

Applications are invited for 2 GRADUATE RESEARCH ASSISTANT POSTS available from February 1. Applicants should have an Honours degree or equivalent in either pharmacology or a biological science. Salaries according to age and experience. The research is concerned with the isolation, characterisation and pharmacological testing of an anti-inflammatory substance present in human plasma.

Further particulars from Professor M. J. H. Smith. Tel.: 01-274 6222, ext. 2446. (568)

CSIRO

AUSTRALIA

DIVISION OF LAND RESOURCES MANAGEMENT CANBERRA, A.C.T.

RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD: ECOSYSTEM ANALYSIS AND SIMULATION

GENERAL: The Division of Land Resources Management with headquarters in Perth, and a professional staff of 55 scientists located in Perth, Deniliquin, Alice Springs, and Canberra, is concerned with research on the effects of land use decisions on the productivity and conservation of land resources. A small but important part of the Division's activities is the analysis and numerical simulation of ecosystems. A powerful Cyber 76 Computer is available in Canberra.

LOCATION: Canberra, A.C.T.

DUTIES: The analysis and numerical simulation of natural and modified ecosystems, including physical, biological, economic, and decision-making aspects. In the course of these duties the appointee would be expected to maintain existing close relations with scientists of other CSIRO Divisions, Australian Government and State Government Departments, and the Australian National University. The appointee would also be responsible for the general oversight of the small group of 4 scientists.

QUALIFICATIONS: A relevant Ph.D. degree or equivalent qualifications, and extensive experience in the field together with demonstrable research ability.

SALARY: Appointment will be made within the salary ranges of Principal Research Scientist or Senior Principal Research Scientist: $A14,882–$A20,005 p.a.

TENURE: The position is available for an indefinite period and Australian Government Superannuation benefits are available.

Applications stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number: 284/16 should reach:

The Personnel Officer,
Australian Scientific Liaison Office,
64–78, Kingsway,
LONDON, WC2B 6BD, by the 8th March, 1974.

Applications in U.S.A. and Canada should be sent to:
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.

(574)

East of Scotland College of Agriculture

invites applications for the following posts:

Head of Animal Production Advisory and Development Department

Applicants should have knowledge of R & D and Advisory aspects of livestock production. Salary scale £4,057 to £5,167.

Animal Breeding Specialist

Applicants should possess postgraduate experience in R & D programmes. Salary scale £2,087 to £3,640.

F.S.S.U. with non-pensionable pay supplement to offset contributions.

Application, by February 7 to the Secretary, The Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG.

(523)

UNIVERSITY OF BRITISH COLUMBIA

DEPARTMENT OF CHEMISTRY

FACULTY POSITIONS

Applications are invited for positions at the rank of Assistant Professor. Applicants should be well-qualified, and those with postdoctoral experience in the fields of experimental Inorganic, Physical or Bio-Physical will be given preference. Date of appointment is open.

Interested applicants should send a full curriculum vitae and the names of three referees to: Professor C. A. McDowell, Department of Chemistry, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5. (556)

PORTSMOUTH POLYTECHNIC
DEPARTMENT OF CHEMISTRY

Applications are invited for a

LECTURESHIP IN MATERIALS TECHNOLOGY

from graduates with a specialised knowledge of the chemistry and application of PLASTICS/PAINTS and other PROTECTIVE COATINGS.

The appointment will be made either at Lecturer Grade I or Lecturer Grade II level according to qualifications and experience.

Salary scales: Lecturer Grade I, £1,660 to £2,847 per annum; Lecturer Grade II, £2,515 to £3,243 per annum.

Application forms and further particulars are obtainable from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Alexandra Road, Portsmouth PO1 2QQ, to whom completed applications should be returned as soon as possible. Please quote reference D40. (533)

UNIVERSITY OF NEW SOUTH WALES

SCHOOL OF ZOOLOGY

LECTURER

High academic qualifications required. Applicants with research interest in some aspect of marine biology, population studies and/or biometry preferred. Duties will include participation in the invertebrate zoology course and undertaking some teaching in the first year biology course. Salary $A8,698 range $A11,982 per annum. Commencing salary according to qualifications and experience.

Details of appointment, including superannuation, study leave and housing scheme, may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close in Australia and London on March 22, 1974. (500)

PLYMOUTH GENERAL HOSPITAL

A science graduate is required to fill a new post based at Plymouth General Hospital for work principally involving saturation analysis and related techniques. Initially it is intended to set up tests of pituitary, thyroid and adrenal function, plus a number of other assays, and there will be some work involving liquid scintillation counting. In addition to the laboratory techniques, the successful candidates will take day to day charge of automatic counting equipment.

Previous experience in this field of work is essential and further details may be obtained by contacting Dr. Janice Went, telephone Plymouth 61003.

Application forms may be obtained from the Hospital Secretary, Plymouth General Hospital, North Friary House, Plymouth PL4 8QQ, and should be returned by not later than 14 days from the appearance of this advertisement.

(537)

UNIVERSITY OF KEELE

Applications invited for two newly created LECTURESHIPS IN GEOLOGY. Applicants should have good geological qualifications with research interests in geochemistry (including sedimentary) for one post, and in geophysics for the other post. Appointment first three points of Lecturers' salary scale (1,929-£4,548 per annum).

Further particulars from the Registrar, The University, Keele, Staffs. ST5 5BG, to whom applications should be returned by February 28, 1974.

(569)

UNIVERSITY OF KEELE

DEPARTMENT OF PHYSICS

Applications invited for post of LECTURER tenable from October 1, 1974. Interest in Biophysics, or physics of macromolecules an advantage. Salary in scale £1,929 to £4,548.

Further particulars and application forms from the Registrar, The University, Keele, Staffs. ST5 5BG, to whom they should be returned by February 25.

(540)

COTTON RESEARCH CORPORATION

PLANT BREEDING AND ENTOMOLOGY OVERSEAS

CRC is a British technical assistance organization with research teams working on the cotton crops of ten countries in Africa, Asia and South America.

Plant breeders and entomologists are in constant demand, and recruits are sought in both fields for appointment in 1974. The work is important and challenging but facilities are often restricted and applicants must have a realistic approach to living and working conditions.

The minimum requirements are a good honours degree, some years of relevant experience and a working knowledge of agricultural statistics. A higher degree would be an asset. Applicants should be citizens of, and permanently resident in, the United Kingdom.

Emoluments include basic salary, overseas and family allowances and an addition in lieu of post-service benefits. Provision is made for housing, leave, passages, baggage and children's education. Initial contracts for up to 4 years are renewable by mutual agreement.

Personal and family details, with a statement of qualifications and experience and the names and addresses of two referees, should be sent to Cotton Research Corporation, 14 Grosvenor Place, London SW1X 7JL. (475)

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOLOGICAL SCIENCES

RESEARCH ASSISTANT

Suitably qualified biologists are invited to apply for a post, financed by the SRC, for work under Professor W. D. P. Stewart and Dr M. J. Daft on microbial pathogens of fresh water algae.

The appointment will be for 20 months at a salary of approx. £1,400 p.a.

Applications, containing the names of two referees and quoting Ref. Est/20/74J, should be sent by March 1, 1974, to The Secretary, The University, Dundee DD1 4HN, from whom further particulars are available. (572)

Chair in Mathematical Statistics

The Chair in Mathematical Statistics at the Royal Veterinary and Agricultural University of Copenhagen is to be filled as per August 1, 1974.

Duties: To participate in both basic training of undergraduates and to undertake instruction of Ph.D. students. It would be desirable for the Professor to be instrumental in a further development of relevant statistical methods and their application within the University in collaboration with the various Departments of the University. Among applicants competent in mathematical statistics precedence will be given to persons with a documented ability to cooperate with researchers in other subjects.

Emoluments: The post is established according to scale 37 of the Danish Government scale of pay plus general allowances for scale 37 plus special bonuses applicable to Professors at Universities.

Form of Application: Application with information about Degrees and academic and pedagogic work must be made to Her Majesty the Queen and submitted to Den kgl. Veterinaer- og Landbohojskole, Bülowsvej 13, 1870 Kobenhavn V. Danmark by the end of February, 1974.

The application, appendices, literary works, and complete lists of publications, as well as a list of appendices must be submitted in quintuplicate.

Further information is obtainable on application to The Registrar at the address given above.

(474)

CSIRO

AUSTRALIA

DIVISION OF CLOUD PHYSICS, EPPING, N.S.W.

RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD: **ATMOSPHERIC PHYSICS**

GENERAL: Current investigations in the Division (located in Sydney, N.S.W.) are concerned with the distribution and properties of atmospheric nuclei, basic and experimental studies of the physical processes which lead to cloud and rain and evaluation of the feasibility of modifying precipitation by cloud seeding.

A position is available in a group investigating the distribution and nature of atmospheric and stratospheric particles.

DUTIES: To investigate the size distribution, chemical nature and sources of those particles on which cloud drops grow; electron microscope techniques for this purpose have been developed in the Division. This will involve calibration and development of sampling techniques for use in aircraft. Field work will be necessary in areas where cloud seeding is proposed or being conducted. The contribution to cloud processes of particles produced by man's activities will also be considered.

QUALIFICATIONS: A Ph.D. degree, or equivalent qualifications, in physics, or an allied experimental field, together with demonstrable research ability. Experience in atmospheric physics, meteorology or aerosol technology would be an advantage.

SALARY: Appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: $A9,380-$A14,290 p.a.

TENURE: The appointment will be for a fixed term of three years.

Applications stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number: 425/10 should reach:

The Personnel Officer, Australian Scientific Liaison Office,
64–78, Kingsway, LONDON, WC2B 6BD
by the 1st March, 1974.

Applications in U.S.A. and Canada should be sent to the Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C. 20036, U.S.A.

(489)

LIVERPOOL POLYTECHNIC

School of Pharmacy

RESEARCH ASSISTANT IN PHARMACOLOGY

Applications are invited from graduates (or those expecting to graduate in 1974) in Pharmacy, Pharmacology, Biochemistry or related disciplines for the post of Research Assistant in the Pharmacology Section of the School of Pharmacy. Industrial experience in biochemically orientated pharmacological techniques woud be an advantage, but is not essential.

The successful applicant will be registered for a Ph.D. of the Council for National Academic Awards, which will be completed in 3 years. Research will be in the field of Psychopharmacology, with special reference to the relationships between changes in brain monoamine metabolism and mood/behaviour induced by hormones and drugs.

Salary will depend on previous experience but will be within the range £1,353 to £1,644 per annum.

Informal enquiries may be made to Dr. Sally R. Tonge, 051-207 3581, Ext. 7. Completed application forms should be returned to the Staff Office, Department (N), Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool L3 9RH, not later than three weeks after the appearance of this advertisement. (526)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)
Denmark Hill, London SE5 8RX

SCIENCE GRADUATE with experience, preferably in tissue culture, immunological methods and cytogenetics, required to work as a Research Assistant in the Genetics Laboratory. The successful applicant will be expected to participate in a project concerned with the diagnosis of cancer. Salary according to age and experience.

Applications in writing with the names of two referees to the Secretary of the Medical School (N), not later than February 14, 1974. (505)

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF HUMAN BIOLOGY AND ANATOMY

SENIOR LECTURER/LECTURER required. Applicants must be medically qualified and capable of teaching topographical anatomy and neuroanatomy to medical and dental students. Research interests in the central or autonomic nervous systems desirable. Excellent research facilities, particularly in the fields of ultrastructure, histochemistry and autoradiography.

Salary scales: Senior Lecturer, £4,368-£5,496; Lecturer, £1,929-£4,548.

Applications (8 copies) with the names of three referees should be sent to the Registrar and Secretary by March 15. Further details may be obtained by writing informally to Professor R. Barer. (369)

CSIRO

AUSTRALIA

DIVISION OF FISHERIES & OCEANOGRAPHY, CRONULLA, N.S.W.

RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD: **MARINE CHEMISTRY**

GENERAL: The Division has a staff of over 40 scientists comprising physicists, biologists, mathematicians and statisticians studying the physical, chemical and ecological characteristics of Australian oceanic and coastal waters. The Division is well equipped with respect to analytical, chemical and oceanographic measuring equipment and has on-line access to a CDC 7600 computer.

DUTIES: To join an interdisciplinary team investigating major factors governing the quality of the aquatic environment. To plan and carry out research on chemical components of marine ecosystems that contribute significantly to the overall ecosystems research strategy.

QUALIFICATIONS: A Ph.D. degree, or equivalent qualifications, with considerable experience in chemical analysis of biological material and natural waters, together with demonstrable research ability. Graduates with a basic degree in agricultural science or biochemistry would be considered for this position, as well as chemistry graduates. Applicants should preferably have had some experience in systems methodology and computer usage.

SALARY: Appointment will be made within the salary ranges of Senior Research Scientist or Principal Research Scientist: $A12,298–$A17,077 p.a.

TENURE: An indefinite or a three years fixed term appointment may be negotiated. An indefinite appointment carries Australian Government Superannuation benefits.

Applications stating full personal and professional details, the names of at least two professional referees and quoting Reference Number: 320/511 should reach:

The Personnel Officer, Australian Scientific Liaison Office,
64/78 Kingsway, LONDON, WC2B 6BD,
by the 1st March, 1974.

Applications in U.S.A. and Canada should be sent to

The Counsellor (Scientific), Embassy of Australia,
1601 Massachusetts Avenue, N.W., WASHINGTON, D.C. 20036,
U.S.A.

(488)

CANADA

DEPARTMENT OF PATHOLOGY

requires a person with research interests in cell injury and having experience in electron microscopy and other cell study techniques. Ample resources, including an E.M. unit, are available for the development of a research programme of the candidate's choice in this subject. While the post is suited for individuals trained in pathology, enquiries are invited from those trained in other fields but active in abnormal cell structure and function studies. Academic appointment.

Write: Department Head, Department of Pathology, Dalhousie University, Halifax, N.S. (471)

UNIVERSITY OF WESTERN ONTARIO

Applications are invited for the position of ASSISTANT or ASSOCIATE PROFESSOR. Responsibilities involve teaching undergraduate and gaduate students and conducting a research programme in biochemistry. Ph.D. and research experience in fields of physical biochemistry, membrane biology, or related areas will be given preference.

Send curriculum vitae and three references to Dr. W. C. McMurray, Secretary, Selection Committee, Department of Biochemistry, University of Western Ontario, London, Ontario, Canada N6A 3K7.

(261)

THE ROYAL DENTAL HOSPITAL SCHOOL OF DENTAL SURGERY

(UNIVERSITY OF LONDON)
32 LEICESTER SQUARE, LONDON, W.C.2

LECTURESHIP IN BIOCHEMISTRY IN RELATION TO DENTISTRY

Applications are invited for the above post which will be implemented in October 1974. Candidates should be post-doctoral preferably with research interests in the connective tissue and/or oral biochemistry fields. Those with experience in conducting lecture and practical classes in Biochemistry for dental undergraduates and graduates will be preferred.

Applications with curriculum vitae and names of three referees should be submitted before February 28 to the School Secretary. (562)

RESEARCH ASSISTANT REQUIRED BY Institute of Cancer Research, Belmont, Sutton, Surrey, to join team studying immunotherapy of human malignant disease. The candidate will be required to measure by *in vitro* techniques the immunological response of patients to malignant cells. Experience in tissue culture essential and experience in immunological techniques desirable. Salary according to experience, in scale £1,770 to £2,418.—Please apply in duplicate to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, S.W.7, giving the names of two referees and quoting Ref. 301/B/500. (564)

UNIVERSITY OF WESTERN ONTARIO

LONDON, ONTARIO, CANADA

Applications are invited for the position of ASSISTANT PROFESSOR IN THE DEPARTMENT OF APPLIED MATHEMATICS, effective July 1, 1974. Applicants should be qualified in Solid or Fluid Mechanics, preferably, but not necessarily, in Solid Mechanics. The current salary floor for an Assistant Professor is $12,300. A contribution towards removal expenses will be made.

Intending candidates should submit their curriculum vitae, together with the names of three referees, to:

Dr M. Zamir,
Chairman, Selection Committee,
Department of Applied Mathematics,
The University of Western Ontario,
London, Ontario,
Canada N6A 3K7.

(513)

TOP GRADE BIOCHEMIST/ CONSULTANT CHEMICAL PATHOLOGIST

The Wessex Regional Hospital Board requires a top grade Biochemist or Clinical Chemical Pathologist for the Basingstoke Clinical Area to take charge of the Clinical Chemistry Service and develop a new highly automated clinical chemistry laboratory in the new Basingstoke District General Hospital.

A suitable candidate will be offered an Honorary Senior Lectureship in Chemical Pathology at the University of Southampton. Duties will include 2 sessions weekly in Southampton for teaching and research.

Application forms and job descriptions available from the Senior Administrative Medical Officer, Highcroft, Romsey Road, Winchester, Hants. Please quote reference 826.

Closing date: February 21, 1974. (490)

THE MEDICAL COLLEGE OF ST. BARTHOLOMEW'S HOSPITAL

WEST SMITHFIELD, LONDON EC1A 7BE
AND

THE LONDON HOSPITAL MEDICAL COLLEGE

JOINT DEPARTMENT OF VIROLOGY

Applications from medical or science graduates are invited for the post of Lecturer in Virology. The post will be based at St. Bartholomew's Hospital. Salary scale (a) £3,012 to £4,668 (Clinical); (b) £1,929 to £4,548 plus £163 London allowance (Non-clinical).

Duties will include assistance with routine hospital diagnostic service, teaching and participation in research work. Further information can be obtained from Dr. R. B. Heath (Tel.: 01-606 7777, Ext. 7213).

Application forms, obtainable from the Secretary of St. Bartholomew's Hospital Medical College, should be returned to the Dean within three weeks from the date of publication of this advertisement.

(484)

UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY

POSTGRADUATE RESEARCH IN BIOLOGICAL CORROSION

Research student required with good Honours Degree, preferably in Microbiology, Biochemistry or Biochemical Engineering, to investigate microbial aspects of the corrosion of aircraft fuel tanks. Applicants should have a knowledge of culture techniques (preferably both batch and continuous) and/or a good background in Organic Chemistry with a strong biological interest.

Applications (no forms) to Professor G. C. Wood, Corrosion and Protection Centre, from whom further information may be obtained (or telephone 061-236 3311, ext. 525). Quote reference JDAM/RS.

(495)

HEREFORD COUNTY HOSPITAL

A basic grade BIOCHEMIST, preferably post-probationary, is required for the Department of Clinical Chemistry at the above hospital. The department is being rehoused and re-equipped in the very near future. The successful candidate will work under the supervision of the Principal Biochemist from whom further details may be obtained.

Applications, giving details of age, previous experience and names and addresses of two referees to the Senior Consultant Pathologist by February 28, 1974. (486)

THE UNIVERSITY OF LANCASTER

DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for three Lectureships within the fields of:

BIOCHEMISTRY

GENETICS

MICROBIOLOGY OF FRESH WATERS

The appointments will be on the scale £1,929-£4,548, and it is hoped the successful applicants will take up the posts by October 1, 1974.

Further particulars may be obtained (quoting Reference L.803) from the Establishment Officer, University House, Bailrigg, Lancaster LA1 4YW, to whom applications (5 copies), naming three referees, should be sent not later than March 10, 1974. (538)

UNIVERSITY OF IBADAN

NIGERIA

Applications are invited for the post of TECHNICAL DIRECTOR IN THE MEDICAL ILLUSTRATION UNIT. Applicants should possess a good educational background and recognised qualifications in Art and/or Photography, and should have considerable working experience in a Hospital Medical Illustration Unit. It is essential that applicants be familiar with medical terminology, and be mature persons with considerable administrative experience. Salary scale: N3,460-N4,060 p.a. (£1 sterling=N1.48.) The British Government may supplement salary in range £850-£950 p.a. (sterling) for married appointee or £350-£450 p.a. (sterling) for single appointee (normally free of all tax) and provide hcildren's education allowances and holiday visit passages. Family passages; various allowances; superannuation scheme; biennial overseas leave.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail not later than March 4, 1974, to the Registrar, University of Ibadan, Ibadan, Nigeria. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (502)

UNIVERSITY OF THE SOUTH PACIFIC

FIJI

Applications are invited for the post of LABORATORY MANAGER IN THE SCHOOL OF NATURAL RESOURCES. Applicants should be a Fellow/Associate of the Institute of Science Technology or equivalent. They should be familiar with equipment and teaching laboratory requirements for Biology, Chemistry and Physics and should have had several years experience as a Laboratory Manager, Chief Technician or Laboratory Superintendent in a multi-disciplinary establishment. Some knowledge of building design and construction and of conditions in a developing country would be desirable. Applicants should have proven ability to supervise personnel satisfactorily and be proficient in safety procedures. The Laboratory Manager has the overall responsibility, under the general direction of the Head of the School, for the ordering and stock-keeping of equipment and chemicals; the organisation of technical facilities within the School; the supervision and allocation of duties of the technical staff, in consultation with Senior Technicians in each discipline; and arrangements for training technical staff.

Salary scale (under review): $F5,500-$F6,900 p.a. (£1 sterling=$F1.88). Appointments are permanent, or on contract for 2, 4 or 6 years. 10% annual taxable gratuity for contract appointment; contributions up to 10% of salary to an approved superannuation scheme; partly furnished accommodation at maximum rental of 15% of salary; various allowances; family passages; regular overseas leave.

Details of the form of application, further particulars and outline terms of service are obtainable from the Registrar (Post 74/1), University of the South Pacific, P.O. Box 1168, Suva, Fiji, to whom completed applications (6 copies) should be returned by March 21, 1974. (503)

UNIVERSITY OF YORK

DEPARTMENT OF BIOLOGY

Applications are invited for the post of TECHNICIAN GRADE 3 to assist in a research project mainly concerned with classification and variability of West African butterflies, though some computational work may be involved. The project is financed by the S.R.C. for a period of approximately 3 years. Salary on scale £1,650-£1,920 depending on experience and qualifications.

Applications, in writing, to the Laboratory Superintendent, Department of Biology, University of York, Heslington, York YO1 5DD, with the names and addresses of two referees by February 22, 1974. (570)

CSIRO

AUSTRALIA

DIVISION OF FISHERIES & OCEANOGRAPHY CRONULLA, N.S.W.

RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD: **OCEANOGRAPHY**

GENERAL: The Division has a staff of over 40 scientists investigating the physical, chemical and biological features including fisheries resources, of marine waters of Australia.

The Division's Water Movements and Properties Group consists of a balanced mixture of physical and chemical oceanographers. The general aim of this Group is a basic understanding of ocean systems in relation to preservation of quality, management of resources, and climatological forecasting. Headquarters of the Division is located at Cronulla, Sydney, with regional laboratories in Perth and near Brisbane. On-line access to a CYBER 7600 computer is available at Cronulla.

The appointee will be located at Cronulla.

DUTIES: To pursue research in the descriptive oceanography of Australasian waters. This will encompass the consolidation of information from a wide variety of observational sources into a comprehensive picture of the genesis, movements and resource interactions of the water masses around Australia.

To collaborate with meteorologists in a study of the possible influence of various water masses upon the climate of Australia, with biologists in the forecasting of variability in fisheries resources, and with marine ecologists in biological studies.

QUALIFICATIONS: A Ph.D. degree, or equivalent qualifications, in marine chemistry or physics with demonstrable research ability and considerable experience in descriptive oceanography. Experience in relating oceanographical information to biological features would also be necessary. Familiarity with computer based techniques for identification of water masses and structural features of the ocean would be highly desirable.

SALARY: Appointment will be made within the salary ranges of Senior Research Scientist or Principal Research Scientist: $A12,298–$A17,077 p.a.

TENURE: An indefinite or a three year fixed term appointment may be negotiated. An indefinite appointment carries Australian Government Superannuation benefits.

Applications stating full personal and professional details, the names of at least two professional referees and quoting Reference Number: 320/505 should reach:

The Personnel Officer, Australian Scientific Liaison Office,
64–78, Kingsway, LONDON, WC2B 6BD
by the 1st March, 1974.

Applications in U.S.A. and Canada should be sent to The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C. 20036, U.S.A.

(514)

UNIVERSITY OF QUEENSLAND

AUSTRALIA

LECTURER IN MICROBIOLOGY

Requirements: A Ph.D. or equivalent in some field of Microbiology. The University is primarily interested in applicants with experience in the study of DNA composition and hybridisation and the mapping of the bacterial genome, but applicants' experienced in other fields of microbial genetics will be considered.

Salary: $A8,698 to $A11,982 per annum.

Other Benefits: Superannuation similar to F.S.S.U., housing assistance, study leave, travelling and removal expenses.

Additional information and application forms are obtainable from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close in London and in Brisbane on **March 30, 1974.** (521)

UNIVERSITY OF QUEENSLAND

AUSTRALIA

SENIOR LECTURER, MICROBIOLOGY AND FOOD TECHNOLOGY

Requirements: A degree in Veterinary Science, Microbiology or Food Technology and appropriate practical and research experience. Duties: Teaching and research in the fields of food microbiology and technology with particular reference to meat hygiene and veterinary public health.

Salary: $A12,268-$A14,308 per annum.

Other Benefits: Superannuation similar to FSSU, housing assistance, study leave, and travelling and removal expenses.

Additional information and application forms are obtainable from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close in London and in Brisbane on February 28, 1974. (509)

UNIVERSITY OF WARWICK

LECTURESHIP IN MICROBIOLOGY

Applications are invited for a Lectureship in the Department of Biological Sciences, with a special interest in microbiology. The appointment will be made within the first four points of the Lecturer scale £1,929, £2,058×£165—£4,368, £4,548 p.a., with FSSU, and is to commence from October 1974.

Further particulars and application forms may be obtained from the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No. 26/7Q/74, to whom completed forms should be returned by February 28, 1974. (515)

SUPERINTENDENT

UNIVERSITY BOTANIC GARDEN
CAMBRIDGE

Applications are invited for the post of SUPERINTENDENT from October 1, 1974. The post carries full responsibility for the practical horticulture work of the garden. The appointment will be made in the grade of Technical Officer, the pensionable scale of stipend being £2,388 a year, rising by annual increments of £165 to £3,378.

Further particulars are obtainable from the Professor of Botany, Botany School, Downing Street, Cambridge CB2 3EA. Applications (6 copies) together with the names of two referees should be sent to the same address to arrive not later than February 27, 1974. (480)

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE OF ANIMAL PHYSIOLOGY

BABRAHAM, CAMBRIDGE CB2 4AT

SCIENTIFIC OFFICER required in the Biochemistry Department to assist with the isolation and characterisation of proteins and sub-cellular fractions. Applicants should have a pass degree, H.N.C., or equivalent. Salary in scale £1,318 to £2,177 per annum according to qualifications and experience. Superannuation scheme with a non-pensionable allowance to offset contributions.

Applications with curriculum vitae should be sent to the Secretary of the Institute as soon as possible quoting reference B.D.20. (561)

UNIVERSITY OF WARWICK

PROFESSORSHIP OF ENVIRONMENTAL SCIENCES

Applications are invited for a new post of Professor of Environmental Sciences tenable from October 1, 1974. Salary in the Professorial range (minimum £5,625 per annum). Superannuation and removal allowances payable.

Further particulars may be obtained from the Academic Registrar, University of Warwick, Coventry CV4 7AL, to whom applications (3 copies) naming three referees, should be sent by February 28, 1974. Please quote Ref. No.: 26/Q/74. (443)

SENIOR ELECTRON MICROSCOPY TECHNICIAN

required for the newly established Neurochemistry Department of this Institute, to work mainly on subcellular fractions used to study synaptic transmission. An initial knowledge of German is not required, since members of the Department are English or English-speaking, but a willingness to learn German is expected.

For full details send short curriculum vitae to: W. Edwards, Abteilung für Neurochemie, Max-Planck-Institut für Biophysikalische Chemie, D-3400 Göttingen-Nikolausberg, Am Fassberg, Postfach 968, West Germany. (476)

UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY

POST-DOCTORATE RESEARCH IN PROTEIN PRODUCTION

Ph.D. preferably in Chemical Engineering, Biochemical Engineering, Biochemistry or Microbiology, to work on an Agricultural Research Council-supported two-year investigation into the production of edible protein from filamentous algae. A knowledge of continuous culture techniques and analytical methods in Biochemistry would be an advantage.

Applications (no forms), to Professor G. C. Wood, Corrosion and Protection Centre, from whom further information may be obtained (or telephone 061-236 3311 extn 525). Quote reference JDAM/PD. (496)

ROYAL FREE HOSPITAL

DEPARTMENT OF CHEMICAL PATHOLOGY

There is a research vacancy for a graduate to work on a project involving bone tissue culture and radioimmune assay techniques. The appointment is to be made as soon as possible.

Salary scale £1,824 to £2,517 plus £126 London Weighting.

Apply in writing to: Dr. M. R. Wills, Department of Chemical Pathology, Royal Free Hospital, Grays Inn Road, London, W.C.1. (479)

UNIVERSITY OF SHEFFIELD

M.SC.TECH. BY RESEARCH

Scientists and Engineers with honours degrees are invited to apply for a bursary (£695+fees), to study the emission of infra-red radiation from exhaust gas plumes.

Please write to Dr P. J. Foster, Department of Chemical Engineering and Fuel Technology, Mappin Street, Sheffield S1 3JD. Please quote ref. R27/G. (497)

POST-DOCTORAL RESEARCH ASSISTANT Required to investigate structure and function of embryonic haemoglobins. Salary and conditions on Whitley Council scale.—Applications, giving details of experience, to the Secretary, University College Hospital Medical School, University Street, London WC1E 6JJ, or for further details ring 01-387 9300, ext. 614, quoting reference ERH/HAEM. (541)

FELLOWSHIPS AND STUDENTSHIPS

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF CANCER STUDIES

POSTGRADUATE STUDENTSHIPS

Applications are invited for two research studentships leading to the degree of Ph.D., tenable for three years. The subject of the research would be some aspect of the relationship between tumour viruses and the host cell genome. The studentships are supported by the Cancer Research Campaign. Good facilities exist for virology, tissue culture, cytogenetics and molecular hybridization. The value of the grant will be £695 per annum, and the conditions of award similar to those for S.R.C. and M.R.C. awards.

Applications with curriculum vitae and names of two referees to Professor D. G. Harnden, Department of Cancer Studies, Medical School, Birmingham B15 2TJ, by March 11, 1974. (531)

AGRICULTURAL RESEARCH COUNCIL

LETCOMBE LABORATORY

RESEARCH STUDENTSHIP

Applications are invited for an Agricultural Research Council post-graduate STUDENTSHIP tenable at Letcombe Laboratory for a period of three years commencing October 1974. The selected candidate will be able to undertake research, leading to a higher degree, within the programme of the Laboratory which is directed to studying the growth and function of plant root systems, and especially the way in which they are affected by soil environment.

Applicants should be British subjects normally resident in the United Kingdom and should have a first or upper second class honours degree in a relevant scientific subject. Full details of the value of the award, and further information on the work of the Laboratory can be obtained from the Secretary, Agricultural Research Council, Letcombe Laboratory, Wantage OX12 9JT, Berks. Closing date for receipt of application is March 31, 1974. (499)

THE ROYAL SOCIETY

SCIENTIFIC INFORMATION RESEARCH FELLOWSHIP

Applications are invited for a SCIENTIFIC INFORMATION RESEARCH FELLOWSHIP for research likely to improve scientific information services. The appointment is tenable for two years in the first instance from October 1, 1974, and may be renewable annually up to a maximum of five years. The stipend will be in the range £3,597 to £4,257 per annum, with superannuation benefits.

Forms of application are available from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, and applications are receivable by February 28, 1974. (557)

IRELAND

FELLOWSHIP IN MARINE SCIENCE

The Pfizer Chemical Corporation, Ringaskiddy, Co. Cork, is sponsoring a FELLOWSHIP IN MARINE SCIENCE tenable at University College, Cork, under a scheme administered by the Fisheries Division of the Department of Agriculture and Fisheries.

Applications are invited from suitably qualified persons to undertake a study of some aspects of the ecology of Cork Harbour.

QUALIFICATIONS: **Essential:** At least a Second Class Grade I Honours University degree in Zoology. **Desirable:** Experience in some aspect of Marine Biology.

The initial starting stipend will be within the range of £1,600 to £1,800 per annum.

Application forms and conditions of service attaching to the Fellowship may be obtained from: the Secretary, Department of Agriculture and Fisheries, Fisheries Division, 3 Cathal Brugha Street, Dublin 1, Ireland. Telephone: Dublin 41121.

Closing date for the receipt of completed application forms is 4 p.m. on Friday, March 29, 1974. (483)

LINACRE COLLEGE, OXFORD

RESEARCH STUDENTSHIPS

Linacre College proposes to make elections to one or two E.P.A. Cephalosporin Studentships tenable for two years in the first instance from October 1, 1974, but with the possibility of an extension for a third year. The value of these studentships will be £695 per year together with approved fees, but may be reduced if the student holds another award or grant; the minimum value of a studentship will be £50 per year.

These awards are open to men or women, but the field of study of candidates is restricted to the Medical, Biological, or Chemical Sciences. Candidates must either have commenced a course of research leading to an Oxford degree in these fields not earlier than October 1973 or be intending to commence such a course of study in October 1974. Successful candidates will be required to become members of Linacre College.

The College also proposes to make elections to two Erin Studentships tenable for two years in the first instance from October 1, 1974, but with a possibility of extension for a third year. These awards are of a value of £100 per year; they are open to men and women and there is no restriction of subject. Candidates must have commenced a course of research in Oxford leading to the degree of D.Phil., B.Litt., or M.A., or B.Phil., or to a Diploma, not earlier than October 1973 or be intending to commence reading for one of these degrees in October 1974.

Candidates for these awards should submit an account of their previous academic work and a brief description of their topic of research, together with the names of two referees to the Principal of Linacre College before March 15, 1974. (545)

POSTGRADUATE STUDENTSHIP

Applications are invited from graduates with 1st or upper second class honours degree. A general knowledge of biochemistry is required, preferably with a special interest in mitochondrial or muscle biochemistry.

The successful candidate will be expected to study the general biochemical properties of red and white, and fast and slow muscles. The project would involve assay of the myoglobin haemoglobin and mitochondrial contents of the muscle, together with a study of their myofibrillar ATP-ase, mitochondrial and Ca-pump activities.

The work is expected to form the basis of an MSc or PhD thesis.

Further particulars of award, allowance, together with a form of application from Secretary, Meat Research Institute, Langford, Bristol BS18 7DY. (508)

UNIVERSITY OF KENT AT CANTERBURY

FACULTY OF NATURAL SCIENCES

RESEARCH FELLOWSHIP IN THE BIOLOGICAL LABORATORY

Applications are invited for a post-doctoral Research Fellowship concerned with physiological and genetic aspects of transmissible antibiotic resistance in bacteria. Bacteriological experience essential, with, preferably, some immunology or microbial biochemistry. Salary initially £1,929 in the range £1,929 to £2,223.

Application forms and further particulars of the post may be obtained from the Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent, to whom completed application forms should be submitted by February 20, 1974. Please quote ref. A23/74. (525)

NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a POSTDOCTORAL RESEARCH FELLOWSHIP supported by the MRC for work on inositol lipid metabolism and diabetic neuropathy. Both animal work and clinical studies will be involved and technical assistance is available. Experience in neurochemistry, phospholipid metabolism or carbohydrate metabolism would be helpful. The appointment is for three years in the salary range £1,929 to £2,388 from a date to be agreed between April 1 and October 1, 1974.

Applications, with curriculum vitae and names of two referees to Professor J. N. Hawthorne, Department of Biochemistry, The Medical School, University of Nottingham, University Park, Nottingham NG7 2RD, from whom further details may be obtained. (362)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF CHEMISTRY

RESEARCH STUDENTSHIP IN POLYMER CHEMISTRY

Applications are invited for a research studentship to study the **Chemical Modification of High Polymers.** The project is financed by the Ministry of Defence and the grant has the same value as a S.R.C. Studentship.

Applications should be addressed to Dr. G. G. Cameron, Chemistry Department, University of Aberdeen, Meston Walk, Aberdeen AB9 2UE, from whom further particulars may be obtained. (535)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF PURE AND APPLIED CHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIPS

Applications are invited from graduates in Chemistry, Physics, Chemical Physics or other relevant disciplines for two S.R.C. Postdoctoral Fellowships.

One will work on the rheological properties of LIQUID CRYSTALS in collaboration with Dr. F. M. Leslie (Mathematics) and Dr. A. J. Hyde (Chemistry). The other will be concerned with the RAMAN SPECTROSCOPY of amorphous polymers in collaboration with Drs. R. T. Bailey and A. J. Hyde (Chemistry). This work forms part of the research programme of the Strathclyde/Glasgow Polymer Centre.

Salaries for both posts will be in the range £1,929–£2,223 p.a. with F.S.S.U., placing according to experience.

Applications (quoting R3/74) should be sent to Dr A. J. Hyde, Department of Pure and Applied Chemistry, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL.

(550)

RESEARCH FELLOWSHIPS

Applications invited

The National Fund for Research into Crippling Diseases (Action Research for the Crippled Child), a voluntary organisation concerned with promoting research into the prevention, cure, treatment or environmental problems of physical disability, invites applications for a limited number of research fellowships. The fellowships will generally not exceed three years and the starting salary will be according to experience and seniority, but will not exceed £3,500 per annum. A small sum for research expenses will be allowed to the department where the work is carried on. Applications from non-medical scientists will be considered.

Applications, including full curriculum vitae, the proposed research programme and a supporting statement from the head of the department in which it is proposed that the research be carried out, should be sent, together with the names of two referees, to the Director, National Fund for Research into Crippling Diseases, Vincent House, 1 Springfield Road, Horsham, Sussex, by April 30, 1974. (536)

UNIVERSITY OF MELBOURNE

DEPARTMENT OF PHYSICAL CHEMISTRY

AUSTRALIAN PAPER MANUFACTURERS LTD.

POST-DOCTORAL FELLOWSHIP IN COLLOID SCIENCE

Applications are invited for a post-doctoral fellowship in Colloid Science, established by Australian Paper Manufacturers Ltd. in the Colloid and Surface Chemistry Group of the Department of Physical Chemistry, University of Melbourne.

The applicant will undertake fundamental and applied research on specific colloidal processes in paper making and related water treatment. The Fellow will be based on the Colloid and Surface chemistry group under the overall direction of Dr. T. W. Healy and close liaison and cooperation with the Research Division of the sponsor company is envisaged. Preference will be given to applicants with experience in the chemistry of collodial dispersions.

The salary will be circa $A8,500 per annum, depending on the experience of the applicant. The appointment is for three years subject to satisfactory progress after the first year. The Fellow is expected to commence as soon as possible.

Applications and confidential reports from two referees should be sent to the Secretary, Graduate Studies, University of Melbourne, Parkville, 3052, Victoria, Australia. Closing date for applications is February 28, 1974. (522)

LINACRE COLLEGE, OXFORD

JUNIOR RESEARCH FELLOWSHIPS

Linacre College proposes to make elections to not more than three Junior Research Fellowships tenable for one year in the first instance from October 1, 1974, and renewable for a second year. Fellowships will be of a minimum value of £100 per year, together with dining rights in College, but the stipend may be increased up to a maximum of £1,100 per year if the holder enjoys no other emoluments. The Fellowships are open to men and women graduates of any university; there is no restriction of subject, but candidates will be expected to pursue research at post-doctoral level.

Applications should be made to the Principal giving details of the applicant's academic record and proposed course of research, and the names of three referees. Closing date for application is April 1, 1974. (544)

UNIVERSITY OF LEEDS

WELLCOME MARINE LABORATORY

Applications are invited for the post of RESEARCH FELLOW at the Wellcome Marine Laboratory which is situated at Robin Hood's Bay. The successful candidate will be expected to pursue broadly ecological research among benthic or pelagial organisms of the littoral or coastal zones. The appointment will be made for one year in the first instance, with possible renewal for a further period of two years. Salary on the scale £1,929 to £2,388.

Forms of application and further particulars from the Registrar, The University, Leeds LS2 9JT (please quote 78/1/D). Closing date March 15, 1974. (554)

THE NUFFIELD FOUNDATION

TRAVELLING FELLOWSHIPS IN MARINE BIOLOGY (TROPICAL AND SUB-TROPICAL)

The Nuffield Foundation offers two Fellowships per annum to enable biologists in U.K. universities to travel overseas (normally for a period of between six and twelve months) to gain scientific experience of inshore marine environments in tropical and sub-tropical regions. Consideration can also be given to applications from physical scientists and from candidates who may be working in institutions other than universities. The primary aim of the scheme is to enliven and to broaden the scope of biology teaching at the tertiary level. The Fellowships are open to men and women holders of U.K. or other Commonwealth university degrees. The value of the awards will be determined on an *ad hoc* basis but will cover fares, subsistence allowances, and contributions to equipment and running expenses.

Applications, for awards to be taken up in 1975, should be submitted by March 31, 1974. Further particulars and forms of application are available from the Nuffield Foundation, Nuffield Lodge, Regent's Park, London NW1 4RS. (264)

DRUMMOND FELLOWSHIP FOR RESEARCH in Nutrition. The Managing Trustees will consider in May 1974 applications for a Drummond Fellowship for Research in Nutrition, normally tenable for two years from October 1, 1974; value within lower part of University Lecturer scale, depending on qualifications and experience; superannuation and expenses grant. Preference given to candidates with biochemical interests in the field of inherited metabolic disease.—Full particulars from Hon. Secretary, Drummond Trust, University College London, Gower Street, London WC1E 6BT. Completed application forms must be received by March 4, 1974. (563)

GRANTS & SCHOLARSHIPS

IMPERIAL COLLEGE

CONOCO SCHOLARSHIP IN PETROLEUM ENGINEERING

available for session 1974/75. Successful applicant will register for 9 months' postgraduate course in petroleum reservoir engineering at Imperial College, leading to degree of M.Sc.

Good honours degree in 2(1) bracket essential in geological, mathematical, physical, or chemical engineering science. Industrial experience preferable.

Bursary of £750 available plus college and registration fees.

Application forms obtainable from Professor W. D. Gill, Geology Department, Imperial College, London SW7 2BP. Closing date April 30, 1974. (549)

THE ROYAL SOCIETY

GOVERNMENT GRANT FOR SCIENTIFIC INVESTIGATIONS

Applications for grants from the first allotment of the Government Grant for Scientific Investigations for the year 1974 should be made as soon as possible on forms of application to be obtained from the Executive Secretary of the Royal Society, 6 Carlton House Terrace, London SW1Y 5AG. No application can be considered which is received later than March 31.

Applicants must be of British nationality, resident in Great Britain or Northern Ireland. Grants may be made for purposes in connexion with the promotion and support of research in pure science other than for personal maintenance or payment of stipends; for the assistance of scientific expeditions and collections; but not in aid of scientific publications. (520)

SHELL SCHOLARSHIP IN GEOPHYSICS

The Royal Dutch/Shell Group of Oil Companies invite applications for a Scholarship in Geophysics. This Scholarship is valued at £750 a year plus fees, for men or women of any nationality who are under the age of twenty-seven on October 1, 1974 and who hold, or expect to hold, a university degree.

The Scholarship will be for a period of two years from October 1, 1974, and, subject to satisfactory progress, may be renewed for a third year. An elected Scholar will be required to pursue a course of training in research in Geophysics at the University of Cambridge and he must be, or must become, registered as a Research Student at the University.

Applications for the Scholarship should be sent to the Secretary of the Department of Geophysics, Madingley Rise, Madingley Road, Cambridge CB3 0EZ, to arrive not later than April 1, 1974. Each application must be accompanied by a statement of the candidate's academic career, his date of birth and the names of two referees. (477)

UNIVERSITY OF LEICESTER

RESEARCH SCHOLARSHIPS

Applications are invited for a number of Research Scholarships in ARTS, SCIENCE (including Engineering), LAW, MEDICINE, and the SOCIAL SCIENCES, tenable at the University for one year initially at a maximum annual value of £695 plus fees. Research Scholars may have the opportunity of acquiring some part-time University teaching experience.

Particulars and application forms may be obtained from the Registrar, to whom completed applications must be submitted by May 1, 1974. (518)

LECTURES AND COURSES

UNIVERSITY OF LEEDS

DEPARTMENT OF EARTH SCIENCES

M.Sc. Courses in

GEOCHEMISTRY
GEOPHYSICS
ENGINEERING GEOLOGY AND GEOTECHNICS

Applications are invited for entry to the above postgraduate courses which lead to the degree of M.Sc. by examination. The courses are for 12 months, beginning on October 1, 1974 and they have been approved for the award of Advanced Course Studentships.

The entry requirements are a 1st or 2nd class Honours degree in the appropriate subject; Geology or Earth Sciences, Chemistry, Physics, Mathematics, Civil Engineering or Mining, or a combined scheme of study which includes at least one of these subjects.

The course in Engineering Geology and Geotechnics is run jointly with the Departments of Civil Engineering and Mining and Mineral Sciences and the course in Geophysics is run in conjunction with the Department of Mining and Mineral Sciences.

Further details and application forms can be obtained from the Secretary, Department of Earth Sciences, University of Leeds, Leeds LS2 9JT. (493)

N.A.T.O. ADVANCED STUDY INSTITUTE ON THE "THEORY AND APPLICATION OF DIFFERENTIAL GAMES"

UNIVERSITY OF WARWICK
COVENTRY, ENGLAND

August 27-September 6, 1974

Organising Committee: R. J. Elliott (Hull), L. Markus (Minnesota and Warwick), P. C. Parks (Warwick).

Speakers include many well known experts in the field.

The number of places is limited, early application is advised and should be not later than May 31, 1974.

Applicants should be mathematicians or mathematical engineers interested in theoretical aspects of control systems and differential equations. Financial assistance for travel and subsistence for successful applicants from N.A.T.O. countries will be available.

For further details and application forms write to: Professor P. C. Parks, Control Theory Centre, University of Warwick, Coventry CV4 7AL, England. (481)

UNIVERSITY OF LEICESTER

M.Sc. COURSE IN EXPERIMENTAL SPACE PHYSICS

Graduates, and prospective graduates, in Physics or Engineering are invited to apply for admission in October 1974 to this one-year course in Experimental Space Physics leading to the M.Sc. degree.

The course of lectures and laboratory work will provide a survey of current researches in Space Physics and will include a comprehensive treatment of the techniques employed in space research. Some industrial studentships will be available and the course is also recognised by the Science Research Council as suitable for the award of its advanced course studentships.

Full particulars and application forms may be obtained from the Registrar, quoting PG5. (517)

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF CERAMICS, GLASSES AND POLYMERS

Applications are invited from physicists, chemists, metallurgists and others with suitable background for RESEARCH STUDENTSHIPS on a wide range of challenging investigations. Grants at S.R.C. rates.

Apply to Professor R. W. Douglas, Department of Ceramics, Glasses and Polymers, The University, St. George's Square, Sheffield S1 3JD (from whom further particulars may be obtained). Please quote reference R28/G. (559)

UNIVERSITY OF LONDON: A lecture entitled "HL-A, The Major Histocompatibility System in Man: A Super Supergene" will be delivered by Professor W. F. Bodmer (Oxford) at 5.30 p.m. on February 13 at University College (Botany Lecture Theatre), Gower Street, WC1. Admission free, without ticket.—Academic Registrar. (507)

International Union Against Cancer

TRAINING COURSE IN CANCER RESEARCH

31st March–12th April 1974

Lausanne, Switzerland

Institut Swisse de Researches Expérimentales sur le Cancer

The Course is intended for junior post graduate students in Biology and Medicine who wish to specialize in Cancer Research.

Special attention will be given to animal husbandry and inbreeding; mouse tumour and transplantation; tumour induction; tumour growth measurements; karyotypic analysis of tumours; tissue culture of tumour cells; immunological considerations; and the preservation and shipment of tumours.

The Course which will be essentially of a practical nature will be given by an international panel of instructors. The teaching will be in English.

There will be no entrance fee but students will be expected to cover their own travelling and living expenses.

Further information may be obtained from: International Union Against Cancer, Rue De Conseil Général, 3, 1205 Geneva, Switzerland.

Applications should be sent to the Union before 1st March, 1974. (571)

INSTITUTE OF CONNECTED EDUCATION

offer prizes for research in pure general science. That is, research in inter-disciplinary sciences, or if and how all sciences are interconnected. Main object of the Institute is to create and sponsor the acceptance of a course of this nature in schools, colleges and institutes.

For further information please apply in writing to: **The Secretary, Institute of Connected Education, 28a Cadogan Place, London SW1X 9RX.** (558)

UNIVERSITY OF ASTON IN BIRMINGHAM

DEPARTMENT OF CHEMISTRY

SESSION 1974-1975

ONE-YEAR M.SC. COURSE IN THE CHEMISTRY AND TECHNOLOGY OF POLYMERS

Some places still remain on this M.Sc. course which has been established since 1964. It provides an understanding of the science and technology of those polymers used in the plastics, rubber, synthetic fibre and surface coating industries. It is suitable for students with a science degree or a similar qualification. Applications from graduates who have spent a few years in one branch of the polymer industry and wish to widen their knowledge would be particularly welcome.

Information and details of Studentships may be obtained from:

Mr. A. J. Lovett,
Department of Chemistry,
The University of Aston in Birmingham,
Gosta Green,
Birmingham B4 7ET.
Tel: 021-359 3611, Ext. 522.

Research places in polymer science and technology, leading to the award of higher degrees, are also available. (555)

PREDICTION OF DISPERSION IN THE ATMOSPHERE

A one-day course of post-experience lectures will be given at IMPERIAL COLLEGE on Wednesday, March 27, 1974.

Further information from Dr. M. J. G. Wilson, Department of Chemical Engineering and Chemical Technology, Imperial College, London SW7 2BY. (534)

UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY

M.SC. IN POLYMER AND FIBRE SCIENCE

This M.Sc. course, which was inaugurated in 1966, combines formal studies with a research investigation and is organised jointly by the Department of Polymer and Fibre Science in the Faculty of Technology (U.M.I.S.T.) and the Department of Chemistry in the Faculty of Science (University of Manchester). The studies are designed to enable a science graduate to obtain an advanced knowledge of the physics and chemistry of polymers. A parallel Diploma course, which omits the research content, is also available.

Applications are invited from Honours graduates (or holders of equivalent qualifications) in chemistry, physics, chemical engineering or a similar subject. Applications will also be considered from persons expecting to graduate next June. The Science Research Council has accepted the course as suitable for the tenure of its Advanced Course Studentships.

Applications and enquiries should be addressed to Professor Geoffrey Allen, Department of Chemistry, University of Manchester, M13 9PL, or to Professor R. H. Peters, Department of Polymer and Fibre Science, U.M.I.S.T., Manchester M60 1QD. (494)

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Electron microscope, Siemens Elmiskop 1 1959

(Serial No. 296)

Additional items include goniometer stage, specimen air lock, plate air lock, electronic battery reference unit for lens stabilisation, coldfinger. The instrument has been regularly serviced by the manufacturer and still meets original specification. For further details apply Mr. A. W. Mortimer, UKAEA, Risley, Warrington. Telephone Warrington 31244, extension 2678. (472)

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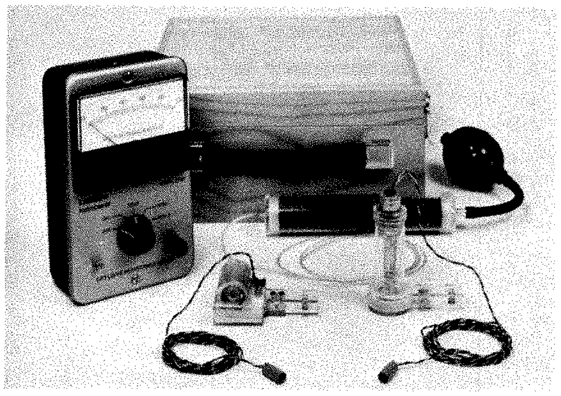

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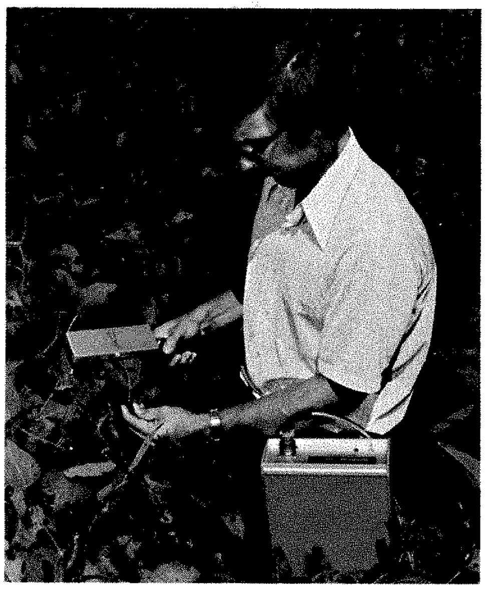

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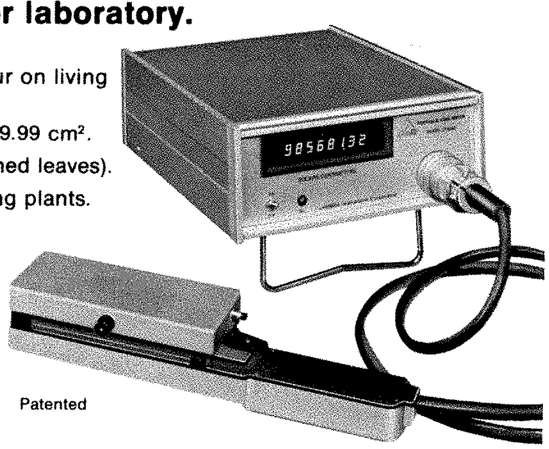

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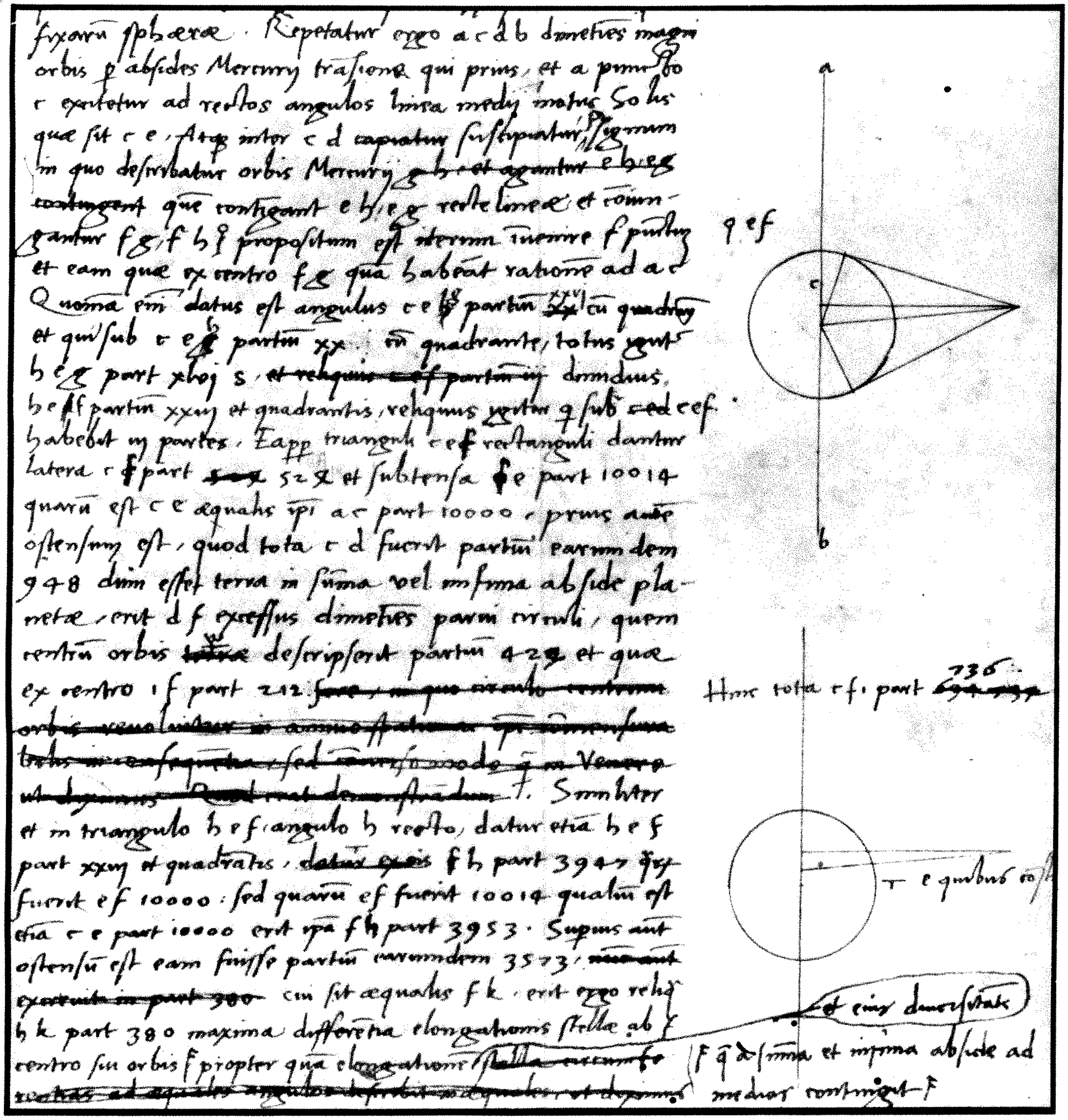

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A journal of exciting biology: Cell

The first issue of **Cell**, a new monthly journal of cell and viral biology from The MIT Press, appeared last month and response has been overwhelmingly enthusiastic. Edited by Benjamin Lewin, formerly the editor of *Nature New Biology*, **Cell** provides a wide-range journal publishing articles of general interest in molecular biology, cell biology, tumor virology and immunology, genetic development and other related areas. Papers are refereed as rapidly as possible and those accepted will be published within twelve weeks.

Cell also includes an extensive review section. In addition to those reviews with the traditional role of discussing a field in some depth, short reviews give conceptual accounts of recent advances in areas of cellular biology, for the benefit of researchers in other areas. Book reviews and discussions of meetings are also included.

The February issue of Cell will include the following articles and reviews:

Viral Hepatitis, the B Antigen and Liver Cancer: A. J. Zuckerman

Integration and Excision of Phage Lambda: M. Gottesman

GUG and UUG are Initiation Codons In Vivo: J. H. Miller

The Molecular Biology of Tumour Viruses, edited by John Tooze: B. Mahy

Altered Metabolism of the Guanosine Tetraphosphate, ppGpp, in Mutants of E. coli: G. Stamminger and R. A. Lazzarini

Continuous Production of Radiation Leukemia Virus in C57BL Thymoma Tissue Culture Lines: Purification of the Leukemogenic Virus: M. Haas

Changes in RNA in Relation to Growth of the Fibroblast. I. Amounts of mRNA, rRNA and tRNA in Resting and Growing cells: L. Johnson, H. Abelson, H. Green and S. Penman

Studies on Amino Acid Control of Cellular Function: V. Morhenn, R. Kram, A. Hershko, and G. M. Tomkins

Translational Capacity of Deadenylated Messenger RNA: E. Bard, D. Efron, A. Marcus, and R. P. Perry

The March issue of Cell will include the following articles and reviews:

Sequence organization of Eucaryotic DNA; defining the unit of gene expression: B. Lewin

Sublines of Mouse 3T3 Cells that Accumulate Lipid: H. Green and O. Kehinde

A Precursor to Hemoglobin mRNA in Nuclei of Immature Duck Red Blood Cells: M. Macnaughton, K. B. Freeman, and J. O. Bishop

Interspersion of Repetitive and Non Repetitive DNA Sequences in the Sea Urchin Genome: D. E. Graham, B. R. Neufeld, E. H. Davidson, and R. J. Britten

A Polymerase Activity forming 5S and pre-4S RNA in Isolated Hela Cell Nuclei: L. McReynolds and S. Penman

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A fuller guide appeared in *Nature* (**246**, 238; 1973).

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Grant Swinger Sees Hope in Energy Crisis

Following is an interview with Dr. Grant Swinger, director of the Center for the Absorption of Federal Funds.

Q. Dr. Swinger, as an ob[illegible] fairs, please [illegible]

[illegible]s. The President has since disbanded the Special Energy Committee, after one of its three members, Mr. Haldeman, became otherwise occupied, and he has subsumed the National Energy Office in a newly established White House Energy Policy Office, which, like its immediate predecessor, [illegible]es on the energy function of the Office of Science and Technology, which was abolished.

Q. Why did he abolish the Office of Science and Technology?

A. Because, as he explained, the expertise that it provided had become sufficiently available throughout the government's departments and agencies to render it unnecessary at the White House level.

Q. Then why did he establish the National Energy Office and then the Energy Policy Office?

A. To provide the White House with the energy expertise that was formerly the responsibility of the Office of Science and Technology.

Q. I see.

A. This also enabled the President to bring in Governor Love of Colorado as Director of the Energy Policy Off[illegible] and Assistant to the President. [illegible] about energy or Washington [illegible] unknown there, he can provide a [illegible] look.

Q. But what about Mr DiBona, who headed the National Energy Office?

A. He benefited from a background similar to Governor Love's, its true. In any case, he remains as the President's Special Consultant on Energy, and is an adviser to the Special Assistant, Governor Love.

Q. What else is happening?

A. The National Science Foundation has established an Office of Energy Policy which will advise the Foundation's Director in his capacity as Science Advisor to the White House.

Q. But hasn't the Director also established a Science and Technology Policy Office to advise him on his role as Advisor?

A. Certainly, but you [illegible] pendent on [illegible]

Foundation's program of Research Applied to National Needs, which, in turn, benefits from advice provided by the National Academy of Sciences in its statutory role as science advisor to the federal government.

Q. Hmm. Does the Science Advisor advise the President on energy matters or does he advise the President's Special Assistant for energy?

A. No. He advises the Secretary of the Treasury, who is the President's adviser on domestic economic affairs. I would point out too, that he also advises the Office of Management and Budget associate director for energy and natural resources.

Q. Then, energy policy is being formulated through NSF's Office of Energy Policy in conjunction with the White House and the Office of Management and Budget?

A. Not exactly. You see, the White House has (continued on page 6)

In Brief

James T. Ramey, who back in 1962 went from the directorship of the congressional Joint Committee on Atomic Energy to membership on the Atomic Energy Commission, has returned to the JCAE as a consultant, following expiration of his AEC term. Though identified as a Democrat and the JCAE's own "man on the Commission," Ramey's departure from the AEC drew an unusually warm goodbye letter from President Nixon.

Henry Kissinger's appointment as Secretary of State reopens the question of the future of the Department's Office of International Scientific and Technological Affairs. Under Herman Pollack, a nonscientist career officer, the Office has thrived as never before, but many State officials still regard it as an odd-ball outfit remote from the Department's major functions and policy-making operations. Kissinger, unlike his predecessors, has shown more than a passing interest in science and technology, and even had his own little scientific "brain trust" while serving at the White House.

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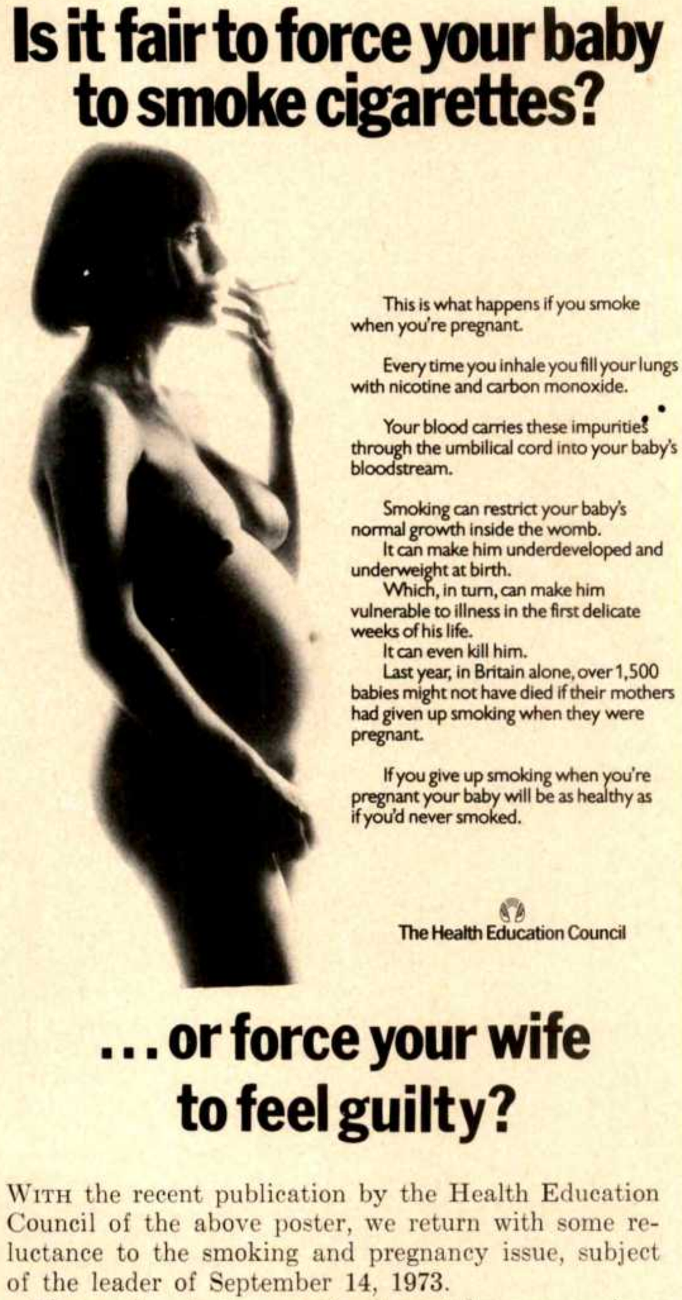


Is it fair to force your baby to smoke cigarettes?

This is what happens if you smoke when you're pregnant.

Every time you inhale you fill your lungs with nicotine and carbon monoxide.

Your blood carries these impurities through the umbilical cord into your baby's bloodstream.

Smoking can restrict your baby's normal growth inside the womb.
It can make him underdeveloped and underweight at birth.
Which, in turn, can make him vulnerable to illness in the first delicate weeks of his life.
It can even kill him.
Last year, in Britain alone, over 1,500 babies might not have died if their mothers had given up smoking when they were pregnant.

If you give up smoking when you're pregnant your baby will be as healthy as if you'd never smoked.

The Health Education Council

...or force your wife to feel guilty?

With the recent publication by the Health Education Council of the above poster, we return with some reluctance to the smoking and pregnancy issue, subject of the leader of September 14, 1973.

The results of a large study showed that non-smokers have, on average, heavier babies than do smokers, and, probably as a consequence, the pregnant mother who smokes has a 30% greater chance of losing her child than does the non-smoker. Furthermore those who voluntarily gave up smoking during pregnancy had 'non-smoker' babies—heavier and more likely to survive.

The question we raised then and we raise again now is that of the advice that scientists should give the public. It is a central question, and one which crops up in many guises whenever scientific work impinges on public policy making. The obvious first reaction is 'if it abates, do it immediately: discuss cause at leisure'. Dr Renwick has recently argued eloquently for greater emphasis on abatement in medical science (**246**, 114; 1973). There is of course still some residual scientific disagreement (as our correspondence showed) about whether it is the smoker or the smoking that leads to lighter babies, but surely with 1500 lives a year at stake the Health Education Council is justified in spending £120,000 abatively on a frontal (well, lateral) assault on the smoking mother-to-be?

Clearly, stopping smoking during pregnancy is a most desirable thing. If the campaign had been simply to encourage those for whom smoking is an optional activity to opt appropriately, one could have few quarrels with it. But the approach is more than suggestive. Words like 'force' and 'kill' are intended to shock and the target is as much the hardened as the casual smoker. The back of the leaflet consists of a guide to stopping smoking that could have been culled from an army manual—from "there's no painless way to stop smoking, no wonder cure" through to "having made the decision to stop, you must stick firmly to that decision". Poor pregnant mother. As if she doesn't have enough kindly advice and isn't already under enough pressure without being instructed to give up what may have been a lifelong habit. If she succeeds, it won't (as they say) be painless. If she fails, the campaign will at least have instilled guilt, and if she fails and then loses her child, who is to explain to her that only one in four of such losses is attributable to smoking?

Half a million leaflets have been distributed to await the pregnant mother. This well-intentioned campaign would have been much more effective and humane if the money had been used on half a million somewhat less scaring leaflets for schoolgirls informing them that starting smoking now could lead to problems in pregnancy. The way to advise the pregnant mother is not through a stark leaflet but through a well-informed doctor or nurse who can assess what sort of approach is suitable for each individual—and possibly avoid words like 'kill'.

100 years ago

We have received some interesting notes of the work done by the eminent Russian explorer, Dr. von Miclucho-Maclay, which we hope to publish next week. Contrary to the advice of everyone, this intrepid traveller and true devotee of Science is determined upon again visiting the east coast of Papua. When his researches here are complete he intends to visit some of the islands of Polynesia and certain parts of the coast of Australia. This, he calculates, will take up five or six years. The Governor of the Dutch East Indies, like a true man of Science, had given Dr. Maclay, for the last six months, roomy and comfortable quarters in his palace at Buitonrovg. It would be well if all in high position would imitate this kind of "patronage."

From *Nature*, 9, 311, February 19, 1874

international news

More planning needed for North Sea oil

Eleanor Lawrence

ALTHOUGH North Sea oil is indisputably the best thing that has happened to Scotland for a long time, the rush to secure sites on which to build drilling platforms has highlighted just how woefully inadequate are present planning procedures.

Only the most extreme preservationists would deny the importance of getting the oil ashore as quickly and as efficiently as possible. As quickly as possible may, however, have quite different meanings to a government facing a worsening economic and political situation and to the concerned individuals and local associations who want full and detailed inquiries to preserve the essential quality of life and environment.

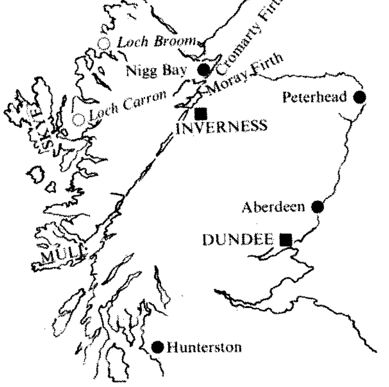
Present planning procedures are ripe for reform say some Scottish conservationists, pointing at the present chaos on the west coast. Dr W. R. Bourne of the University of Aberdeen says that present procedures are wasteful and inefficient and that the only winners at the moment are the lawyers who seem to be *de rigeur* at every inquiry. Most of the sites on the west coast are extremely isolated and far from expert help, so each case must be fought separately and at great expense by the local community.

The present system of separate public inquiries is throwing an increasing burden on the slender resources of local people and local conservation societies. According to Dr Bourne, money that is urgently needed for positive conservation work, to buy land and develop nature reserves and to clear up the effects of pollution, is being diverted in a steady stream into the pockets of the lawyers. To the development companies the long months of uncertainty, waiting for a decision, can also cost money and sometimes results in lost orders.

Last week Mr Gordon Campbell, Secretary of State for Scotland, previewed proposals for a Bill to be presented to Parliament in the near future. This would enable the government to speed North Sea oil ashore by compulsory purchase of the necessary land, which would then be leased to the development companies. These unprecedented measures seem to have been precipitated both by the energy crisis and by the impasse that has been reached at the Loch Carron site on the west coast of Scotland, near the Kyle of Lochalsh. The government promises that it would use its powers only in limited cases, mainly to speed up the building of the deep water concrete drilling platforms which are apparently essential to the oil effort.

These platforms have to be built in deep sheltered water with a nearby shore site. Such sites are rare in Britain but there are several around the north-west coast of Scotland, notably Loch Carron, Loch Broom and Dunnet Bay in Caithness. The Secretary of State for Scotland said that the Bill would permit an early start on work at Loch Carron, probably this summer instead of next year as had been expected if permission had been granted after the inquiry.

An added dimension to the battle at Loch Carron is that the site is owned by the National Trust for Scotland, held 'inalienably' for the people of Scotland. A special Bill in Parliament would in any case have been needed to allow development on such a site and the government's proposals will give them blanket powers over other such sites in any part of Britain.



○, Sites under consideration for development; ●, sites where development is already well advanced.

The government promises that it will take basic resposibility for the site and Mr Campbell promised that there would be provision in the Bill for eventual restitution and restoration of the site. But, as Dr Bourne points out, this becomes a mockery if the government will not allow sufficient time for detailed reports of the present conditions, both social and environmental, to be prepared. At Loch Carron, which will probably be the first victim of the new legislation, no satisfactory reports of the natural history have been prepared says Dr Bourne. Very little is known about the area as yet but reports coming in at the end of last year suggest that the Kyle of Lochalsh is probably one of the main feeding grounds of the eider duck, a characteristic sight in Scottish waters. Flocks about 2,000 strong have been reported and if building goes ahead the eider duck will certainly be dispossessed.

Professor C. H. Gimingham, professor of botany at the University of Aberdeen and secretary of the University's Environmental Liason Group fears that, despite government promises, the new proposals will not allow proper time for objection and will only reveal the complete lack of any overall planning for oil development in Scotland.

Under the present procedures, once a development company has found a site, it seeks planning permission in principle from the local County Council. The applications are then considered by the council, which should allow time for objection. If overwhelming objections are received the decision is deferred and a public inquiry set up at which the objectors put their case. Public inquiries can drag on for many months, as in the case of Loch Carron and eventually the Secretary of State for Scotland has to step in.

One serious drawback of the public inquiry system as it stands, says Professor Gimingham, is that only one case can be dealt with at a time. They are, therefore, completely unsuited to the present situation on the west coast which urgently needs an overall planning policy. An instance of the sad situation which obtains at the moment is the case of Dunnet Bay in Caithness. Dunnet Bay is designated as an area of outstanding natural beauty. Last year the American Chicago Bridge group applied for permission to build deep water oil drilling platforms there. Local residents and the Natural Conservancy fought the proposals but lost to the development company's appeals to the national interest and the need for urgency. A few weeks after permission had been granted the company announced that it was pulling out of Dunnet Bay to a site in Ireland, where 'geophysical conditions' are more favourable, and planning permission is expected to be granted in a matter of weeks. This has made people not unnaturally wary of further appeals to the national interest by both developers and government. The permission to develop remains, of course, and may be taken up at any time.

Professor Gimingham considers that the involvement of National Trust land at Loch Carron is especially worrying and could set a dangerous precedent. In his opinion, a proper planning policy for the west coast should have been able to avoid this.

The Nature Conservancy is also investigating whether the National Nature Reserves which it owns or administers will be covered by the new proposals. The lack of an overall planning strategy is also stressed by Mr R. Goodier of the Scottish Nature Conservancy. Local planning departments have not been expanded to meet the unprecedented demands now placed on them. The present crisis is coming at just the wrong time, he said, as Scotland is entering its reorganisation of local government. A massive injection of new planning talent is needed, he said, but how this is to be achieved without by-passing the essentially democratic procedures at local level is a problem.

The Nature Conservancy opposed the plans to develop at Dunnet Bay and now will probably have to fight its case again when the next development is proposed. Despite the expense and time involved the Nature Conservancy obviously wishes to fight such proposals in the interest of Scottish conservation generally. A proper plan for Scotland would make its task and that of the many other organisations much easier.

Science journals in a prices jungle

John Hall

THE rising prices of scientific journals, combined with the proliferation into ever narrower specialities, is currently a matter of concern to university librarians and all who find themselves faced with subscription rates which bear little relation to the rates they budgeted for when they first subscribed.

The question of cost-effectiveness is an almost impossible one to answer for scientific journals. Not only do journals have very varied reputations and quality standards but the cost of a journal is often an elusive quantity. The advertised prices for a set of journals in 1973 and 1974 are shown in the table. Some journals sell at a single price, others have privileged rates for individuals and yet others published in the United States (not included in the table) have page charges that authors are expected, but not required, to pay. These latter are clearly good value in Europe because Europeans traditionally do not have page charge budgets and thus get a subsidised journal.

The 1973 price per page of learned journals (excluding those such as *Nature* which carry advertising and thus can subsidise their editorial pages with their advertising revenue) is roughly £14 per thousand pages. But when a reduced subscription is available for individuals or society members the price for libraries can be doubled. So, presumably, it is the libraries who subsidise the bargains with which individuals can line their offices.

The question of price rises is not a simple one, as subscribers are asked to pay in advance for an item the quality and quantity of which is not guaranteed to last the year. Thus a modest or zero price rise can reflect a publishing decision to produce less or take less care over it. On the other hand an abstracting journal, riding on the back of a still expanding literature, may have no option but to charge more.

A record breaking increase of 261% in the price of *International Pharmaceutical Abstracts* (published last year by the American Society for Hospital Pharmacists at £16.60 and this year at £58.20) was one of a series of price rises which have prompted the Association of University Teachers (AUT) to launch an inquiry into the costing of science publishing. The AUT has taken a batch of suspected offenders to independent publishers, who are to be asked for realistic estimates of production costs. The union's general secretary Mr Laurie Sapper suspects that certain publishers may be making excessive profits (not a specially untrusting notion, since author's copy for these journals comes as free as God's air) and if the independent costing exercise bears out the suspicion he plans to confront the publishers with the evidence of their greed. Which is all very well, except that the publishers might be expected to know already the extent of their profits and to have reconciled their consciences to an appropriate figure. Unfortunately there is no British Standard specifying the demarcation between excessive and reasonable profit.

There is, however, no denying that the profits from scientific publishing generally look pretty healthy, with some falling into the positively exuberant bracket. Alec Anderson, librarian of Heriot-Watt University, Edinburgh, surveyed price increases of scientific books and journals in his library and found that during the autumn term periodicals cost on average 23.5% more than in the previous year. Apart from the famous increase decreed by *International Pharmaceutical Abstracts,* he quoted the United States *Current Contents: Life Sciences* with an increase of 90% (£48.30 to £86.95); the American Chemical Society's *Chemical Abstracts* (£850 to £1,110); and the *Transactions of The American Association of Electrical and Electronic Engineers* (£252 to £321). Lesson: learned societies may be no more averse to a fast buck than is the commercial operator.

The Library Association has published a price index for periodicals (100 in 1970) which now stands at 147.6; it warns that the index could reach 185 within a year. And with books and periodicals rising in price at something like one and a half times the rate of university costs generally, one or two consumers have balked at the increases. *The Times Higher Education Supplement* had Salford University Librarian Charles Bubb talking about large libraries being vulnerable to milking by publishers and calling for an inquiry into the increased diversion of taxpayers' money to this area of spending. In the same issue the librarian of the University of Birmingham Kenneth Humphries identified the problem of the increased influx of European publications caused by the floating of the pound. Most of these imports were a third more or even double their price three years ago.

A more specific attack on the quality of the products which flood the market was mounted in a letter from an international group of chemists, sent to *Nature* and other journals in the field. British professors Jack Linnett (University

TABLE 1 Comparison of journal prices
(in pounds sterling; concessionary prices in brackets)

Journal	Number of pages	Price	
		1973	1974
Deep Sea Research (Pergamon)	1150	34 (6)	34 (6)
Geophysical Journal (Royal Astronomical Society)	2000	50 (8)	90 (8)
Journal of Fluid Mechanics (Cambridge Univ. Press)	5000	57.50	57.50
Journal of Molecular Biology (Academic Press)	7000	99	141.30
Journal of Physics (Institute of Physics)	2300	39 (10)	42 (10)
Philosophical Magazine (Taylor and Francis)	2900	43	47

of Cambridge) and G. Wilkinson (Imperial College, London) are among eleven academics who point out that the rash of new chemistry journals appearing subsist on high subscription rates rather than publication charges from the authors. They believe that some of the new journals, with a vested interest in building up volume to maintain themselves, have refereeing standards which are lax, to say the least (a certain journal in the field of inorganic and nuclear chemistry has "minimal refereeing standards", and contains "stuff which ought never to see the light of day", says Professor Wilkinson).

The chemists see over-specialisation encouraging the proliferation of journals and being in turn encouraged by them at a time when there should be more communication between scientists working in different areas. They urge, as a quick way of getting the message over to publishers, that scientists should ask their libraries to think twice about subscribing to new commercial journals, and themselves refrain from publishing in them. Somewhat idealistically, they talk of the need for an international agency to examine the founding of new journals, and further suggest that national chemical societies (though they be far from perfect and their track records not always shining bright) should set up "an impartial mechanism for evaluating the need for a new journal and require that criteria for assuring the level of quality is met". This committee's concerns would include "a set of criteria for refereeing practice, statistics about rejection rates, criteria for terminating a journal, restrictions on language of national origin of work, page charges, and so on."

The Chemical Society's Director of Publications, Dr L. C. Cross, confirms the complaints of the group and sums up the overall problem of over-specialisation and the haphazard division of new knowledge into packages of narrow interest in the following terms: "This has merely resulted in ever multiplying costs to the consumer and greater profits to the producer. Such results are inevitable because the package is not being altered fundamentally, only the label is being changed, with results that any housewife would predict".

Another group of scientific authors and editors, including Dr Monty Finniston (*Materials Science*), Professor J. P. Hartnett (*International Journal of Heat and Mass Transfer*) Professor N. Kurti (*International Monographs of Solid State Physics*), and Sir Harold Thompson (*Spectrochimica Acta*) lamented over soaring prices in a letter to Sir Walter Coutts, chairman of their publishers, Pergamon Press Ltd. They also proposed a remedy which, a sceptic might remark, was not guaranteed absolutely to reverse the trend.

"We are alarmed" (they said) 'at the regular annual exorbitant price increases on books and journals, not only by Pergamon but also by other publishers. These augmentations have practically eliminated purchases of publications by private individuals. Libraries, with fixed or reduced budgets, are facing impossible choices between urgently needed publications. Also we deplore the fact that resulting from these price increases students are buying fewer and fewer books for their personal use. Here we need the kind of imaginative leadership that Robert Maxwell can provide in order to reverse disastrous pricing trends in the publishing industry and to help meet these other difficulties".

On the strength of this recommendation (and of rival claims for the merits of a counter proposition) Mr Maxwell was asked by *Nature* whether Pergamon was specially culpable over the issues at stake and what, if anything, he proposed in order to make life more bearable for subscribers.

In the first place, says Mr Maxwell, Pergamon's total of 142 scientific journals represents a growth of less than 5% over the past two years. "There is a growing number of people doing research in a growing number of subjects and because of the pressure on existing publication machinery it is inevitable that new journals emerge to meet these new demands. These chaps have a point about proliferation but in no case do we, the publishers, initiate a new journal; not a single one. A group of scientists will write in and say they feel there is a need for a journal in a certain area, so we talk about it and carry out a detailed, world-wide survey, and if there is a genuine need then we go on from there. But really, the actual proliferation has slowed down immensely".

Naturally enough, Mr Maxwell defends the standards of the referees used by Pergamon. Like the editors and editorial boards of his journals, the refereeing is "of the highest standard". Sometimes, he adds, a paper rejected by a Pergamon publication will turn up in the journal of a learned society, although this is not to say that in a vast output of 20,000 papers a year there is not "an occasional slip-up if a referee has not been as careful as he ought to have been".

As for the cash angle, Mr Maxwell is in scientific publishing because he likes to be of some help to science and, not having been to university, he does

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Dispute halts abstracts scheme

Colin Norman, Washington

A DISPUTE between the American Institute of Physics (AIP) and the London-based Institution of Electrical Engineers (IEE), has brought to a temporary halt an attempt to rationalise the production and marketing of physics abstracts. It seems that the IEE, for financial reasons, has abrogated an agreement worked out between the two organisations last summer, and as a consequence, the AIP has terminated the marketing of IEE information products in the United States—a service which the American society has been performing for the past five years. The disagreement came to light recently when the executive directors of the societies traded public statements on the matter, but both have expressed the hope that a new arrangement can still be worked out.

The cost of producing abstracts of physical articles has shot up in the past few years, with the result that *Physics Abstracts*, the key IEE service, has increased in price from \$12 in 1967 to \$380 in 1974. It is thus circulated chiefly to institutions rather than to individuals. The AIP abstracting services, however, are geared at least partly to the needs of individual subscribers, and in that respect the two services are complementary and compatible. Moreover, since more than 25% of the IEE abstracts are taken from papers published in the primary journals of the AIP, there is clearly considerable scope for cooperation in the production of the abstracts.

Against that background, officials of the two societies worked out an agreement in June last year under which the AIP would supply the IEE with computer-readable abstracts of articles published in AIP journals. The agreement would also have given the AIP considerable influence over the content and design of IEE abstracts, and the British society would have reimbursed AIP for the service. The arrangement would have permitted multiple use of each computer tape, thereby cutting down production time and costs of abstract journals. IEE officials later felt that they were getting a raw financial deal, and reneged on their commitments, whereupon the AIP discontinued marketing IEE products. IEE abstracts are now marketed in the United States by the Institution of Electrical and Electronics Engineers (IEEE).

the best he can by seeing that the work of scientists is disseminated. The profit motive is not uppermost in his mind, and such profit as accrues is useful chiefly to ensure that the overall operation runs efficiently and as a subsidy for prestige loss-leaders like *Leonardo* (bridging arts and science), *Archives of Oral Biology* (for dentists) and *Marcellia* (The International Journal of Phytopathological Morphogenesis and Cecidology).

Among the numerous examples of subsidised services to science which Mr Maxwell says he can name off-hand is the Russian translating service which, although reduced after its first five years of operation, still produces of the order of 20,000 pages a year. The company's last published report showed a profit in the region of £200,000 a third of which came from books. (Trading profit for the year ended September, 1973, was expected to be £292,000.

As a sign of his good intent, Mr Maxwell instances his plans for *Tetrahedron*, a top organic chemistry journal with some 5,000 subscriptions (mainly at the library rate of £102 a year, for which a library receives something like 12,000 pages of information). As an experiment, *Tetrahedron* is to be circulated in the form of expanded abstracts. Thirty papers in this form will occupy 60 printed pages, whereas the full papers would need 300. The net result is a saving of 75% to 80% in costs to the subscriber, who simply writes for the detailed version of such papers as appeal to him, at no more than the photocopying cost. He also plans to cut subscription costs by developing a new typesetting technique for scientific material by harnessing the computer to the traditional archival type of journal; and when the annual rise of 10% in the volume of published literature makes it impossible for libraries to house any more magazines, he plans to be a leader in the field of microfiche and microfilm reproduction. Yes, he says, the portable microfiche reader will eventually oust the paper magazine. Which is all very fine with McLuhan, no doubt, but not much of an answer to the immediate problems of Alec Henderson, Charles Bubb, Kenneth Humphries and a cast of thousands of their library users.

Soviet journals have their problems too

from our Soviet Correspondent

The catalogue *Gazety i Zhurnaly SSSR* (Periodicals of the USSR) for 1974 lists some 600 scientific and technological titles (excluding abstract journals), more than 500 of them in Russian, with Ukrainian—30 titles—as the next most prolific language. According to an article in *Pravda* by Candidate of Technical Sciences R. Ivanov (December 21, 1973) this plethora of specialist information is proving more and more difficult to digest, even on the assumption that a given reader will require to scan only the literature of his own speciality. A survey of 25% of the Soviet specialist journals, Ivanov claims, reveal some significant deficiencies.

Although it has been obligatory since 1967 for all scientific and technical publications to be accompanied by abstracts, only half of the journals studied did include them. Moreover, when abstracts occurred they had frequently not been revised by the editors with their intended readership in mind but were left in the unsatisfactory state in which they came from the authors, who write down as an abstract "Whatever God puts into their souls". Ivanov also criticises the practice of certain journals of printing the abstracts on narrow strips of coloured paper, bound into the front or back of the journal—this may save paper but wastes everyone else's time. Best of all, he maintains, is the practice of printing the abstracts on detachable, filable cards, which saves "many thousand of man hours". (One assumes that the Soviet reader, consulting the journal in a library, would never be so antisocial as to remove such an abstract to his private collection—or is there some security system to prevent this?)

Other major criticisms are the waste of paper at the end of articles (which could be utilised for announcements of forthcoming books, conferences and so on) and the absence in some 80% of the sampled journals of a submission date indicating the age of the material. Seventy-five per cent of the journals ignored the practice ("a sign of high culture") of putting the title, number and date of issue of the journal on every page—thus causing specialists much delay in hunting through their collections of photocopies.

Faced with this abundance of journals, the increasing use of computer indexing and the physical and logistic problems of storage, Ivanov suggests not only such ideas as the introduction of standardised Union-wide identification symbols, and the printing of the abstract and bibliography together for simultaneous scanning (some of which problems are already under consideration by working groups) but the proposal, possible only in a very centralised system, that a single, unified physical format should be adopted for all scientific and specialist journals published in the Soviet Union for greater convenience in shelving.

NIH leaders attacked and defended

Colin Norman, Washington

The longstanding dispute between officials at the National Institutes of Health and their superiors in the Nixon Administration has moved on to new battlegrounds—the correspondence columns of the Washington *Post*. The disagreements, which stem chiefly from the fact that the Administration has been attempting for the past year or so to centralise control over biomedical research policies in the Department of Health, Education and Welfare and the Office of Management and Budget, came to a fresh head recently when Dr John Sherman resigned as deputy director of NIH. He cited a number of intangible reasons for his departure, prominent among which was a complete lack of understanding between officials at NIH and administrators in the Department of Health, Education and Welfare (see *Nature*, **247**, 172; 1974).

The Washington *Post* then criticised HEW officials in an editorial, a move which stung Dr Charles C. Edwards, Assistant HEW Secretary for Health, into firing off a letter to the newspaper, in turn criticising the "inadequate leadership" at NIH. Not surprisingly, several scientists at NIH drafted a reply to Edwards's letter, circulated it around the NIH campus for a couple of days during which it gathered 460 signatures, and dispatched it to the *Post* last week.

The *Post* editorial had called on the Nixon Administration to give NIH the autonomy that it has had in the past, to restore its "world-wide reputation for excellence". But Edwards argued in his letter than an institution which spends $2,000 million a year cannot be completely insulated from budgetary and managerial control. He also maintained that confidence in NIH has "diminished in the past through inadequate leadership and a misguided sense of the place of research in the nation's efforts to solve its health problems."

What really stung NIH scientists, however, was a reference in Edwards's letter to the fragmentation which has been taking place at NIH, particularly the increased autonomy that has been given to the National Cancer Institute. "If the NIH leadership had been more receptive and responsive", he argued, "we might not have witnessed the removal of the cancer research effort from the administrative control of NIH, a move that threatens the further dissolution of biomedical research efforts".

What Dr Edwards seems to have forgotten is that when Congress was debating the National Cancer Act, the NIH leadership fought a battle within the Administration to prevent the National

Cancer Institute from being given autonomy. The move was partly successful because the Act was amended at a late stage in the House of Representatives to keep the cancer institute at least partly within the structure of NIH. At that time, however, the Administration had lent its official public support to a bill which would have set up the National Cancer Institute as virtually an independent body.

The NIH scientists, in their reply, state that "with Dr Edwards, we deplore the fragmentation of the NIH, but it is essential to recognize that this fragmentation was opposed by the NIH administration". The nub of their argument about management control of biomedical research is that "we fully appreciate the importance of political and economic considerations in the process by which the nation decides how much is to be spent on biomedical research. But whatever that sum may be, we believe that the nation will reap the largest benefit if scientific and medical considerations govern the programs and administration of the NIH".

So far, thanks partly to the public airing of their grievances and support from influential members of Congress, NIH scientists have been relatively successful in their efforts to maintain at least a semblance of autonomy for the institute from management control by HEW and OMB. In the process, however, the three top officials who ran NIH during the first four years of the Nixon Administration—Robert Q. Marston, NIH Director, Robert Berliner, NIH Associate Director for Science, and Sherman—have either been forced out or have resigned to take less frustrating jobs.

When is a forgery not a forgery?

John Hall

CURIOUSER and curiouser, remarked at least half a dozen scholarly gentlemen who found that they had stepped out of the grim reality of a February night in London and into the topsy-turvy realm of Vinland. Falling through the portals of the Royal Geographical Society they learned on the one hand that the renowned map of Vinland was drawn in twentieth century ink, and on the other that this did not necessarily mean that it wasn't a genuine work of fifteenth century scholarship illustrating a Norse voyage of discovery to North America.

Turning aside from the richest collection of medieval documents in the world, George Painter made his way from the British Museum's research department to Kensington Gore in order to explain that there was no mistaking the workings of a *bona fide* medieval mind in the cartography, the linguistics and the scholarship of the map which is now the property of Yale University. ("O.K. here's your hundred thousand to buy the thing", Andrew Mellon is reputed to have said, "but if it turns out a forgery, well just don't come calling for anything ever again". Right now he must feel justified in stamping his foot.)

Meanwhile, looking up from his microanalytical mass spectrometer, burly, close-cropped Walter McCrone, of McCrone Associates, Chicago, delivered himself of the opinion that the likelihood of a pigment of the crystalline size and shape of that found in the Vinland Map ink being used in a map of AD 1400 'could be compared with the likelihood that Admiral Nelson's flagship at Trafalgar was a hovercraft.'

Only time will tell, held Painter, how we might arrive at a scenario admitting that a fifteenth century manuscript might remain genuine in spite of its being drawn in an anatase pigment containing titanium dioxide particles first manufactured only fifty years ago. Leaving aside time travel, the prospects for such a scenario seem to fall into three categories. First, suppose somebody happened on the Vinland Map, thought its outline had become too faint for comfortable scanning, and dashed over it with modern ink. Sorry, says McCrone, but there's no sign of further pigmentation below the titanium dioxide ink.

Suppose then that an ingenious scribe, scraping about for a new line in inks, decided to use the very compound which wasn't used again for that purpose until about 1920? No way, says McCrone. Naturally-occurring titanium would contain impurities such as iron and manganese: what's more, the titanium dioxide used was a precipitate, the shape and size of whose particles were indistinguishable from those used in anatase.

All right, says Painter. Textual evidence indicates that the version we have is a copy of earlier versions: is it not possible then that the present version is simply one more copy, a modern one, made from a genuine version of the map, if at several removes? Not even the implacable McCrone can gainsay this possibility: it is not within the realms of spectrometry. Without any sort of material support, the thesis doesn't look al that powerful, but Painter points out that the chemical analysis of the ink is simply one piece of evidence, which has to be weighed alongside considerations such as the quality of the scholarship and the thinking embodied in the map These aspects are not quantifiable, and may seem less tangible than a scientific analysis, but they nevertheless add up to evidence, and deserve to be weighed equally with McCrone's findings.

Painter's hopes for a future reconciliation of the chemical evidence and the scholarly hunch is heartwarming, but it must be reported that his is a voice crying in the wilderness, and the weight of opinion seems to be that the jig is up for the map's supporters. Another of the British Museum's research team, Mr A. D. Baynes-Cope, has had his doubts for some years, and told Yale as much when they asked in 1968. The thing has been scrubbed, as if in attempt to give it a weathered look, and the worm holes in the vellum have been patched in an amateurish way, says Baynes-Cope. The British Museum's own craftsmen could have made a better forgery.

Scholars being what they are, nobody is prepared to say exactly who was the Vinland Map's Mr Big. The name of a European professor of canon law, one Jelic, is bandied about, but as he died in 1922, that wouldn't have left him all that much time to do the job after the introduction of anatase inks. One thing's for sure, and that is that the forger (assuming the thing was forged) didn't make a fortune out of his work. The map was sold for only 3,500 dollars as recently as the late fifties, and even McCrone is bound to admit that the effort was worth considerably more than that. His theory is that maybe some gifted academic drew the map for his private amusement, and that it only assumed importance, and cash value, when it was discovered after his death.

Either way, no real harm has been done anywhere outside the Mellon Foundation's cheque book (if they were the anonymous donors. If not, *pace* Andrew Mellon). Another of the British Museum's sceptics, Dr Helen Wallis, points out that at best the map would have been significant evidence that the Vikings had a good working knowledge of the North American coastline fifty years before Columbus set sail. At worst it amounts to an interesting forgery which, incidentally, has prompted a work of scholarship (*The Vinland Map and the Tartar Relation*, by Skelton, Marston and Painter) which has turned out to be the standard work in that area of study, regardless of the map's worth.

Health risk in PVC manufacture?

THE British Factory Inspectorate has begun discussions with industry to decide what action may be necessary to protect workers from a possible health risk arising from the use of vinyl chloride monomer (an intermediate used in the manufacture of PVC). The talks follow news that an American PVC manufacturer investigating causes of death, among its employees discovered four cases of rare liver cancer which may have been linked to the use of the monomer. The US Department of Labor has arranged a hearing to examine the extent of workers' exposure to vinyl chloride monomer.

The American deaths were at the works of B. V. Goodrich, and in England the death of a 71 year-old former process worker at ICI is being investigated in order to establish whether or not it was caused by long exposure to the fumes of the monomer. ICI employs 1,200 workers in PVC production, and about 380 of these have been warned of possible risks in their particular work.

Vinyl chloride monomer was first used in plastics manufacture in Germany in the 1930s, and in the early days of its use in Britain and the United States a threshold limit value (TLV) of 500 parts per million was observed because of the fire and explosion hazards involved. However, an Italian study in 1971 showed that rats exposed to vapour with a concentration of the order of 3,000 parts per million tended to develop cancer, and a current TLV of 200 parts per million is observed in industry.

The TUC's medical adviser, Dr Robert Murray, has been visiting plants throughout the country in order to consult with producers and advise workers. The difficulty in arriving at a safe level of exposure to the monomer lies in the fact that a carcinogen risk cannot be quantified as rapidly as a fire or a toxicity hazard, says Dr Murray. Furthermore, the organ most likely to be affected by the monomer is the liver, whose condition is not easily diagnosed by conventional methods of examination.

The immediate priority is to attempt to achieve a really low TLV (say, of 50 p.p.m.), to seek mechanical alternatives to the autoclave swabbing which is the main source of contact, and to make sure that nobody enters an autoclave without air-line apparatus.

"What you've got to say to the workers", says Dr Murray, "is that the risk is small: that apart from the risks involved when he drives his car, and eats too much, and drinks too much, here is another risk which he has got to live with. The production of PVC is too big an operation to be stopped entirely because of this hazard, so what we must do is reduce exposure as far as possible while pressing ahead with the epidemiology".

In Washington, Tony Mazzocchi, Legislative Director of the Oil, Chemical and Atomic Workers Union, said that the union is now in the process of identifying those of its members who have been exposed to vinyl chloride monomer, and that they will be examined for signs of liver damage. He said that there is at present no basis for the statement that there is little risk from exposure to the chemical, and predicted that in the United States at least, the matter is is likely to lead to a long battle. The Oil, Chemical and Atomic Workers Union has recently held a long strike against the Shell Oil Company in an attempt to secure a strong health and safety clause in work contracts.

DMSO cut down to size

Miranda Robertson, Chicago

A CONFERENCE held recently in New York to discuss recent investigations with dimethyl sulphoxide (DMSO) may herald the beginning of the end of the continuing story of this controversial drug. A lipid solvent exploited commercially in household cleaners, it enjoyed a burst of popularity in the early 1960s as a miracle drug active against almost everything from arthritic pain to mental retardation until it was banned by the Food and Drug Administration (FDA) in 1965 on the basis of toxicity tests on animals. Now once again it is the subject of clinical investigation but this time on a more sober note.

In spite of a report by the National Academy of Sciences (NAS) last year, which essentially endorsed the decision made by the FDA in 1965, thirteen laboratories now have authorisation from the FDA to look into the effects of the drug on, for example, scerodерma, strains and sprains, and bursitis, in man.

For although the NAS committee, set up in 1972 at the request of the FDA after a public outcry, concluded that profound scepticism was justified, it found the criteria for testing the drug on humans unduly rigorous. Nor is the evidence for its toxicity so terribly alarming. When applied to the human skin (which it penetrates with remarkable rapidity and ease), it causes irritation and blistering in about 50% of cases. In animals, it produces reversible changes in the lens of the eye after the administration of quite unrealistically large doses.

Toxicity apart, there are two main reasons for the bad odour into which the erstwhile wonder drug fell with the authorities. Nobody to this day has the slightest idea how DMSO produces any one of the diverse pharmacological effects claimed for it, for one. For another, it literally gives off a bad odour, within minutes of administration, which not only seems likely to enhance any placebo effect on the patient but makes normal control procedures, such as double blind trials, extremely difficult. Since many of the claims rest on small but significant statistical differences between treated and control (or differently treated) groups, this constitutes a serious criticism—especially in the absence of any rationale which might be provided by an understanding of the mechanism of action of the drug.

In the view of the NAS committee, however, none of this is adequate reason for refusing investigation on man where controlled animal experiments show that there is a possibility of relieving incapacitating pain or saving life. The latter possibility seems to arise in the treatment of neurological injuries, an area which has not yet been given investigatory clearance by the FDA.

Work on a monkey model at the University of Chicago suggests that DMSO may be more effective than urea (the current drug of choice) in relieving compression on the brain due to subdural haematoma, a common and often fatal sequel of head injuries from any cause. The improvement, according to Dr J. de la Torre, is measured not only in somewhat reduced mortality but in decreased neurological damage to the survivors. There is some evidence in this case that the effects of the drug depend partly on a diuretic action and partly on a more mysterious influence on oxygenation.

How the treatment, which has been extended in animal tests to impact injury of the spinal cord and experimental 'stroke', would emerge from the exigencies of a real neurological emergency remains to be seen. Nor is it yet clear whether DMSO will eventually find a useful and respectable place in the pharmacist's therapeutic repertoire; but it seems likely that with its emergence into the cold light of properly controlled investigation, its days as a panacea are over.

Britain backs seafloor search

To encourage British participation in deep sea mining for manganese nodules, the British Government has offered Rio Tinto Zinc and Consolidated Gold Fields Ltd up to £830,000 towards their participation in an international consortium examining the floor of the Pacific Ocean. The money will be repaid if the project leads to commercial production, and the companies have also agreed that British consumers will have first option to purchase a proportion of any metals produced. The project, therefore, is seen as a means of allowing British companies to break into a new technology, and also in the long term as a possible new source of metals (like copper, nickel and cobalt) for British consumers.

The consortium consists of the Kennecott Copper Corporation (United States), RTZ and consolidated Gold Fields, Mitsubishi Corporation (Japan) and Noranda Mines Ltd (Canada). Its programme will include work on the nature and location of deposits of manganese nodules under the Pacific Ocean and investigation of methods for working, transporting and extracting useful metals from them. The programme is expected to last five years and to cost more than $50 million.

Manganese nodulues occur naturally on the beds of all oceans, generally at depths greater than 1,000m: the richest metalbearing nodules have been found in the Pacific at depths of about 5,000m. The content of the nodules varies greatly but the usual range for deep sea nodules is manganese (12% to 34%), copper (0.1% to 1.5%), cobalt (0.1% to 0.3%) and iron (1.6% to 18%).

correspondence

Embryos and Ethics

SIR,—According to Dr Anne McLaren's review (*Nature*, **247**: 121) of "The Mammalian Fetus *in vitro*", Professor R. A. McCance "regrets that the Advisory Group recommended that foetuses of more than 20 weeks' gestation should be considered viable, since he feels that this creates a 'shadowy period of 8 weeks' during which abortions can be performed, yet the use of the abortuses for experiments may become illegal".

But the *Infant Life (Preservation) Act 1929* defines destroying any viable fetus as the crime of 'Child Destruction', unless done "in good faith for the purpose only of preserving the life of the mother". It continues ". . . evidence that a woman had at any material time been pregnant for a period of 28 weeks or more shall be primâ facie proof that she was at that time pregnant of a child capable of being born alive". But it nowhere says that before 28 weeks the child can be presumed not to be viable, and nowadays a healthy baby of 24 weeks may well live under intensive care.

So if there are any prospects of viability at all a pregnancy may only be terminated to preserve the mother's life, and everything possible must still be done to save the child after delivery. A doctor who fails in this duty, by allowing experimentation or otherwise, would at the very least face a charge of manslaughter by neglect, if at autopsy the baby was found to have been capable of surviving, given proper care.

There have been a few reports of survival from well under 24 weeks, though the real reason for setting a limit at 20 weeks is to allow for mistakes over dates and so on. But, in spite of a wide spread belief to the contrary, the 28th week has no special significance, and anyone experimenting with an abortus at any age near to viability does so at his peril. Viability is a biological fact, not a matter merely of legal definition, and there can be no 'shadowy period" during which the child may be presumed inviable by reference simply to dates, either for abortion or for any other purpose.

Yours faithfully,

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Adolescents and science

SIR,—I feel that an important aspect may be missing in the interesting research made by A. Ahlgren and J. H. Walberg (*Nature*, **245**, 187; 1973).

The attitude towards science among adolescents could have changed for the worse because they may have been taught in a way that gives them the impression that science cannot be understood.

Physics has become so abstract that it is very difficult to teach it so that it remains comprehensible for children and yet up to date in its fundamental principles. For instance, it is very difficult to teach relativity at an elementary level and it is practically impossible to do the same with quantum mechanics. Therefore one either teaches classical mechanics and later says that this is only a first approximation, or tries to begin with relativistic quantum mechanics. The latter is evidenly impossible in the present state of affairs. The former easily gives the child the feeling that he is being cheated or at least that the real thing is utterly beyond comprehension. A really epoch making book on 'Modern Elementary Physics' is badly needed—but who is going to write it?

Chemistry is even worse off, especially in the United States and in countries like Israel where we are copying what they do. It is very often taught in the wrong order. Instead of making it plausible why we think matter is composed of atoms and molecules and why we think they are as large as they are, teachers begin with electrons, nuclei, neutrons without even trying to make their existence plausible. The result is a hopeless mess. Less intelligent children just repeat the story like parrots while the more intelligent ones despair. Then chemistry is built on these shaky foundations with a not much more stable superstructure. England is much better off in this respect than many countries. A professor at the Technical University in Stockholm told me also that the decreasing number of chemistry students is probably due to this way of teaching in high schools.

Biology is often ruined by introducing biochemical concepts not necessary at that level, and which quite certainly cannot even be understood. Presenting the formulae of sugars and amino acids before the elements of chemistry have been taught in the chemistry course is evidently absurd. A very intelligent 17 year old boy in New York told me that he has to write a paper on the chemical foundations of genetics, with nucleic acids and so on involved, without even an elementary knowledge of organic chemistry. The Krebs cycle is also one of the preferred subjects. Any half-way intelligent child feels that he is being bluffed and suspects something wrong in science at large.

We shall have to stop bluffing children and build elementary science courses on solid, understandable foundations if we want intelligent adolescents to turn their preference towards science again.

Yours faithfully,

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news and views

Meteorites which 'bounce' off the Earth

BRIGHT fireballs shooting across the sky are often reported in the press. Meteorite collectors become very excited by these reports because careful analysis of the sightings enables them to calculate the trajectory of the incoming particle and to predict the impact point. The brilliant meteor observed over the western United States and Canada in the early afternoon of August 10, 1972 (see page 449 of this issue of *Nature*) however, would have disappointed them because it did not fall to ground but, in fact, 'bounced' off the Earth's atmosphere and flew off into space again. But it did not disappoint Rawcliffe of the Aerospace Corporation, Los Angeles or Barthy, Li, Gordon and Carta of Aerojet Electrosystems Corporation, Azusa who fortunately had a satellite-borne near-infrared radiometer in orbit over the United States at that time and detected the radiation from the meteorite. The meteorite path was found to have a perigee of 58 km, and, by assuming that all the loss in kinetic energy was transferred to thermal radiation from the meteorite, these workers found its mass to be 10^9g. Assuming it to have the density of iron, its equivalent diameter turns out to be 4 m.

From the ground (see *Sky Telesc.*, **44**, 269; 1972) it was seen as a blazing ball of fire, a metallic bluish-white in colour (like a carbon arc or welders torch) moving slowly ($\sim$15 km s^{-1}) across the sky, leaving behind a train which persisted for about an hour and looked rather like a smoke trail. The brightness of the fireball was intermediate between that of the Sun and the full Moon, the apparent magnitude being roughly -18.

Fortunately meteorites of mass 10^9g are uncommon—recent flux estimates indicate that one should hit the Earth every 10 to 20 yr. If this meteorite had hit the ground it would have produced a crater with a diameter of about 200–300 m. The failure of this meteorite to impact, however, is a much rarer occurrence and can only happen to the larger meteoroids (mass greater than a few grams). The 'bouncing' meteoroids enter the atmosphere almost tangentially and an estimate can be made of their frequency by comparing the cross-sectional area of the Earth plus its atmosphere with the area of an annulus of thickness one scale height ($\sim$ 7 km) surrounding the Earth at the height of the meteor region (100) km). This indicates that only 0.2% of the meteoroid flux will bounce, the remaining 98.8% falling to ground.

Rawcliffe *et al.* are not the only scientists to have recorded a 'bouncing' meteorite. On November 11, 1968 a camera mounted on the payload of a 'Hibal' balloon at 31 km above Queensland, Australia photographed a tangential meteor trail. Bigg and Thompson (*Nature*, **222**, 157; 1969) concluded that this meteorite consisted of four sizeable pieces of solid material together with a vast number of tiny dust particles.

It is thought that the effects of grazing meteorites have been observed by the micrometeoroid detector on the HEOS 2 Earth orbiting satellite. Hoffmann, Fechtig, Grün and Kissel (COSPAR, Konstanz 1973) report that this satellite from time to time encountered localised dense swarms of micrometeorites. These swarms were produced, according to Kaiser (University of Sheffield), by the fragmentation of grazing meteorites.

Returning to the report by Rawcliffe *et al.* it would be interesting to see estimates of the amount of energy lost by the meteorite due to frictional drag in the atmosphere. At 58 km, the meteorite perigee, the mean free path is about 0.02 cm and a shock wave could possibly have been produced. If this had occurred, the meteorite would have lost energy to atmospheric turbulence and the authors estimate of its size would have to be reduced. Only one of the ground reports recorded any sound. This was from an observer in British Columbia, where the meteorite was leaving the atmosphere, who reported a faint rumbling 2 to 3 minutes after it had disappeared.

D.W.H.

●

Light and plant disease

THE effect of light on the predisposition to, and subsequent manifestation of, virus diseases in plants has been studied for many years. Bawden and Roberts (*Ann. appl. Biol.*, **34**, 286; 1948) found that plants grown for long periods at low light intensity or subjected to periods of darkness before inoculation were more predisposed to virus infection and often produced more primary local lesions and/or became systemically infected, and thus diseased, in a shorter time.

Virus-infected plants often show more severe systemic diseases when grown in poor light, though subsequent work has shown that many viruses multiply more rapidly in plants that are grown at their optimum light intensity and photoperiod. Shorter photoperiods immediately after inoculation can reduce or delay local lesion formation, whereas high humidity often enhances the development of symptoms in infected plants (Yarwood and Fulton, *Methods in Virology*, **1**, 237; 1967).

Kirkham, Hignett and Ormerod reported recently (*Nature*, **247**, 158; 1974) that the response of cucumber plants to the inoculation of a virus was reduced by interrupting daylight with intermittent 2 min periods of darkness. The authors suggest that the reduction in the formation of local lesions on the inoculated plant was the result of a change in the hormonal complex within the plants. This change could have been stimulated by the intermittent dark periods, especially as the total time of light and dark were equal in all experiments so that the amount of photosynthate was equal in all plants.

Kirkham *et al.* did not, however, study the numbers of virus particles colonising the plants and multiplying within them. Nor was any observation made later to record total numbers of local lesions formed. Thus there is no information on whether interrupting daylight has an effect on initial colonisation of the plant by the virus or whether formation of local lesions was merely delayed and not inhibited. The effect of intermittent lighting on the multiplication and translocation of the virus or the subsequent systemic disease that this virus induces in cucumber was not mentioned.

The East Malling workers studied one virus in one plant and related the plant responses to those induced by two fungal pathogens in other plants growing in conditions of intermittent light. The hypothesis that plant hormones, stimulated by the intermittent light, influence plant disease responses does not take into account the many other physiological systems within plants that are photosensitive and related to disease response. Light intensity and photoperiod can influence cytochrome and enzymatic systems as well as many other biochemical and metabolic activities. The proposed hypothesis would have more credance if the experiments described had been markedly reversed or altered by the addition of plant hormones.

It is also well known that many bacteria and fungi are themselves photosensitive so that changes in photoperiod and light intensity may have a pronounced effect on their pathogenicity as well as the plants' response. Thus it is premature to equate fungal pathogenicity or the severity of a fungal disease with a hormonal response in the plant induced by intermittent light.

It seems likely, however, that further studies along these lines may provide a better undersanding of the complex of plant and pathogen and disease induction. Research workers studying the development of diseases or the assay of fungicides should now be acutely aware of the importance of incident light as well as the other environmental factors such as temperature and humidity, in order to obtain reproducible results.

From a Correspondent

Evolution in action

IF current views on evolution are correct, it must be the case that on occasions a gene changes so as to produce an enzyme with an altered function. It is therefore a challenge to experimentalists to produce such a change by selection in the laboratory. The obvious approach to this problem would be to keep a bacterial population capable of using substrate S, and also capable of using a related substance S^1 but with low efficiency, on the latter substrate, and at the same time to expose the population to a mutagenic agent. Bacterial populations do adapt to such circumstances, but they usually do so by producing enormous quantities of the original inefficient enzyme rather than by evolving a better one. Typically, a 'constitutive' mutation takes place in the system regulating the activity of the gene, so that the gene is permanently switched on; in this state, up to 20% of the bacterial protein may consist of this one enzyme.

Betz, Brown, Smyth and Clarke (*Nature*, **247**, 261; 1974) recently summarised some experiments in which an understanding of the mechanisms of gene regulation have made it possible to bring about in the laboratory the evolution of a new enzyme specificity. The bacterium was *Pseudomonas aeruginosa*, and the substrates were amides used as a source of nitrogen. Normal strains can use acetamide and propionamide; their enzyme acts with very low efficiency on the 4-carbon compound butyramide, but the bacteria cannot utilise this substrate, because butyramide does not induce the production of the enzyme, and in fact acts as a competitive inhibitor of its production. The enzyme is inactive on the 5-carbon compound valeramide.

Betz *et al.* obtained several new strains, including some capable of utilising valeramide, by way of two intermediate steps. The first step was a constitutive strain C11, obtained by selection on formamide—a substrate used with low efficiency by the species. Strain C11 cannot grow on butyramide, because the gene producing the enzyme is repressed by this substrate. From strain C11 a mutant B6 was obtained with a much higher activity on butyramide, but still retaining its activity on acetamide. A third mutational step, starting from B6, gave rise to a class of mutants able to utilise valeramide for growth. A number of other enzymes were obtained by this and other routes, including enzymes active on phenylacetamide.

For biochemists, the existence of a group of very similar enzymes with different specificities may be a useful tool in studying mechanisms of enzymation. For evolutionists, it is both important and reassuring to know that a new enzyme specificity can be produced by very few mutational steps. It is also interesting that each step could in certain circumstances be selectively advantageous, so that there is no need to postulate a long 'random walk' before a new function is achieved.

From a Correspondent

New theory of evoked responses

THERE is a kind of computational equivalent of Parkinson's law, that computation expands to fill the space available. Putting it another way the computer seems to be seen by some as a helpful amplifier of the seriousness and scientific value of research; and at best as a guarantee that the frontiers of knowledge really are being rolled back. In this context it is hardly surprising that the computer has become one of the most significant, and indeed symbolic, denizens of laboratories undertaking research in neurophysiology and psychology. The suspicious might wonder whether the uses of the computer in its symbolic role are quite as well thought out as they ought to be.

The particular function that computers usually play in neurophysiological and psychological research is that of doing statistics on various biological signals, a typical instance being the averaging of electroencephalogram (EEG) activity following the application of stimuli. The argument is that the shape of the electrical response to the stimulus is not discernible in the single recorded trace, because it is buried in uncontrollable spontaneous activity which irritatingly obscures the actual response to the stimulus, but at the same time also obligingly provides the requirement for a computer. By recording the responses to several dozen stimuli, digitising and summing the resultant waveforms in a computer, the consistent features of a response will add together. As the sum of these consistent features grows larger, the inconsistent fluctuations tend to sum to zero, and the signal stands out dramatically from the noise. The procedure seems both logically straightforward, and with the advent of small computers, even indispensable for serious research on the nature of the electrical responses of the brain to stimulus events. Sayers, Beagley and Henshall who are perhaps better versed than many on analysis of biological signals analysis have, on page 481 of this issue of *Nature*, gone directly, if somewhat iconoclastically to the heart of this matter by quite rightly questioning the apparently unassailable logic of this, by now standard, procedure.

Sayers *et al.* point out that underlying the averaging technique is the idea that the electrical response to a stimulus is added to the background of spontaneous activity, so that summing the responses actually selects out this additive component. But this, they declare, is an unexamined assumption, and upon examination, it turns out, a mistaken one. They conclude that what the stimulus probably does is to reorganise already existing components of the spontaneous EEG pattern, and not provoke a new electrical event superimposed upon it.

They cast the problem into the Fourier domain. The

Fourier theorem states that a signal which can be described as varying with time can be alternatively described, without loss of information, as the sum of a set of sinusoidal waves, of specified frequencies, amplitudes and phases. (Phase can be thought of as the time, or relative time, of occurrence of some conspicuous point, for example, the maximum in these waves.) Thus frequency analysis of a signal consists of discovering which sinusoidal frequencies exist in the signal and what amplitudes and phases each of these various sine waves has.

What Sayers *et al.* have shown is that if the supposed response to a stimulus (in their case an auditory one) is compared with a similar length of EEG before the stimulus, then the average power (the square of the amplitude) of the frequency components in the response is not actually any different from the average power in a pre-stimulus interval. If the response were an event superimposed on the spontaneous activity, it would clearly add amplitude (and hence power) in the spectrum. That it does not (and the experimenters display a comparison between the amplitudes of the ten principal frequency components of EEG following supra-threshold and sub-threshold stimuli), seems to indicate that the stimulus must reorganise the phases of pre-existing frequency components of the EEG. This has the effect of making an averaged response apparently stand out from a background, but for a quite different reason than that usually assumed.

In the phase data, the phases of the ten principal sine wave components of the total signal in supra-threshold responses were found to be closely specified with respect to one another and the specification was consistent over a number of runs. In the EEG following sub-threshold stimuli on the other hand, the starting points of all the various component waves measured in different runs were scattered uniformly and apparently randomly over the whole range. Thus a stimulus did seem to have the effect of imposing a specific organisation on the phases of existing frequencies.

To test their conclusion, Sayers *et al.* used the reconstituation technique suggested by the Fourier theorem. They found the amplitudes of the frequency components in sections of spontaneous EEG activity (with no stimuli) from their subjects. Then they ordered the starting points of synthesised sine waves at the different frequencies according to the discovered phase relationships of these frequencies in the post-stimulus signal. The synthetic signals thus produced bore a strong resemblance to 'real' responses, and indeed supported a high coefficient of correlation with them. Reconstituting the other way round, that is to say by finding the phase relationships in a length of spontaneous EEG and then using these to specify the starting points of a set of sinewaves whose amplitudes were found from an actual response, produced a pattern quite different from the post-stimulus records: and one which had virtually zero correlation with them.

This conclusion is the opposite of that derived from the assumption underlying the conventional use of post-stimulus time averaging. It suggests a much more appropriate way of analysing EEG responses, as well as a quite new concept of how they arise. For those researchers who use the evoked response of the EEG simply as an indication that following a stimulus something does actually happen inside the subject's head, this result will probably be of not much significance. For those whose study of evoked responses is any more searching, however, this is a result that can hardly be ignored, even if it does require confirmation in circumstances different than those described by Sayers *et al.*

from our Experimental
Psychology Correspondent

Heliocentric theory in China

from a Correspondent

By the end of 1973 most historians of astronomy had had their fill of the life and work of Copernicus. A report of a quincentenary celebration meeting held in China last June, however, does add a new slant to the innumerable papers already published on the topic (*Scient. Sin.*, **16**, 364; 1973).

It begins in classical style with a quotation from the works of Chairman Mao: "What is correct invariably develops in the course of struggle with what is wrong. The true, the good and the beautiful always exist by contrast with the false, the evil and the ugly, and grow in struggle with the latter . . . Copernicus' theory of the solar system and Darwin's theory of evolution were once dismissed as erroneous and had to win through over bitter opposition." This sets the scene for a discussion of the struggle for acceptance of the heliocentric theory in China.

The initial step is to show that ideas of the Earth's motion existed in China long before the birth of Copernicus. It is eventually admitted that most of the passages offered in proof of this contention must actually be construed as referring to rotation of the Earth, rather than to its revolution round the Sun. (One or two of the quoted extracts require the eye of faith even to discern terrestrial rotation.) These passages, however, enable the authors to claim that the two concepts of a stationary Earth and an Earth in motion had coexisted since ancient times, as had the struggle between them. This struggle was so prolonged because, "until production, commerce, geographical discovery and astronomical observation developed to a new stage, and the new class force in society emerged on the political arena, a scientific heliocentric theory could not have been fully verified".

With the advent of the Jesuits in China, heliocentric views arrived from Europe, but only in a veiled form since the Roman Catholic church was opposed to the concept. "Since", however, "they intended to use science and technology as a means to win the confidence of the Chinese, the Jesuits had to make their transmission of scientific knowledge through the test by scientific practice. In the field of astronomy, they must make their forecast of celestial phenomena tally with actuality. Thus, the objective laws of nature compelled them to deviate from their usual belief and seek assistance from the works of Copernicus, Galileo and Kepler."

In the early eighteenth century, the arrival of instruments employing the concept of a heliocentric system—two armillary spheres from England are specially mentioned—forced the Jesuits on the defensive. But knowledge of the existence of these spheres, and of other relevant material, was kept within a limited circle. The crucial date for a more general dissemination of the heliocentric theory in China only came in 1859. (The significance of this date for European science is not mentioned.) In that year, the first Chinese translation of a modern astronomy text—Sir John Herschel's *Outlines of Astronomy*—made its appearance. By the end of the nineteenth century, the Copernican hypothesis had become sufficiently well known in China to be referred to in popular ballads.

Clearly, the history of the heliocentric theory holds great attraction for the Chinese as an exemplar of the ideology of struggle. But this Copernicus meeting has more significance than that: it provides a further indication that China is gradually moving back into the world scientific community. More still, it is admitted that China has long lagged behind the Western world in science, and still does so today; that this deficiency is something that must remedied. The final words of the report are worthy of note in this connection. "China has fallen behind a great length in science . . . to compare with advanced world level, there is still a wide gap. We are still a developing country. Steps have just been taken to the study of the afore-mentioned significant theoretical problems of science. In the spirit of 'Making the past serve the present and foreign things serve China', we will learn from the people of all countries. Like Copernicus, we will have the courage to break paths where none have gone before and to scale heights yet unclimbed. We will strive to catch up with and surpass the advanced world level in the near future to make a greater contribution to humanity."

Paradox of Earth's core resolved

from our Geomagnetism Correspondent

ALTHOUGH convection in the Earth's mantle is now widely accepted among geophysicists as a strong working hypothesis, it took a long time for this happy state of affairs to be reached from the original proposal of the concept. By contrast, the credibility of convection in the outer core has always seemed much greater and less likely to be eroded by expressions of doubt. To a large extent this is because the fluidity of the outer core was demonstrated by seismology many decades ago, whereas the acceptance of mantle convection requires belief in the more difficult and recent concept of plastic or semi-fluid flow in a solid or near-solid. But core convection also gained credibility by the necessity of explaining the origin of the geomagnetic field. The need for mantle convection depends on the acceptance of continental drift and sea-floor spreading, acceptance which was gained only with a struggle and is even now not quite universal. The existence of the Earth's magnetic field, on the other hand, has never been in doubt, and an origin other than core convection has never proved viable.

Of course, core convection is still an article of faith rather than a proven phenomenon because the existence of fluid, rotating or not, does not in itself imply the presence of convection. Even so, geomagneticians have been somewhat dismayed recently to find Higgins and Kennedy (*J. geophys. Res.*, **76,** 1870; 1971) and Kennedy and Higgins (*J. geophys. Res.*, **78,** 900; 1973) purporting to show that the core is stable against thermal convection. For convection to occur in the core, the adiabatic temperature gradient throughout must be less than the melting temperature gradient, for if this is not the case the required instability cannot develop. Moreover, neither can there be convection if the actual temperature is above the melting temperature and the actual temperature gradient is less than the adiabatic gradient, for convection is then less effective than conduction as a heat transfer mechanism. That the actual temperature throughout the outer core is indeed at or above the melting temperature can hardly be in doubt because the outer core is fluid. It is thus usual to assume that the actual temperature is higher than the melting temperature everywhere except at the inner core-outer core boundary where the temperatures are equal, supposing the boundary to represent the solid-liquid phase change of the core material.

If convection occurs, and because it is a very efficient heat transfer process, the actual temperature at all points will be very close to the adiabatic temperature —and the situation generally assumed hitherto is that both of these curves lie above the melting point curve. Higgins and Kennedy, on the other hand, revised the melting temperature of pure iron (the supposed principal core constituent) and the adiabatic temperature throughout the core, and concluded that the adiabatic temperature curve lies below the melting point curve. The core is still fluid, of course, and so the actual temperature curve is still above the melting temperature curve. But now there is no convection, and the actual and adiabatic temperature curves lie on opposite sides of the melting point curve.

It would be wrong to suggest that geomagneticians have been seriously concerned about all this; so sure are they of the reality of core convection that they have generally been content to sit back and wait for someone to point out the flaw in the Higgins-Kennedy argument. Their wish has now apparently been granted by Frazer (*Geophys. J.*, **34,** 193; 1973). As Frazer points out, Higgins and Kennedy are on shaky ground right from the start because their melting curve is for pure iron, whereas the core almost certainly contains a proportion of lighter elements. The precise effects of these lighter constituents are not known except that they are certain to decrease the melting temperature; but in any case Birch (*Geophys. J.*, **29,** 373; 1972) has concluded that in the present state of knowledge the melting temperature of iron at core pressures cannot be given with an accuracy greater than ± 500 K.

Higgins and Kennedy are thus less secure than they might seem because of the old and common problem of uncertainty in core parameters. But Frazer goes much further in claiming that the Higgins-Kennedy argument is not valid even if their melting temperature curve is accepted because the assumptions made in their calculation of the adiabatic gradient are demonstrably inappropriate to the core; for example, one of the two methods used by Higgins and Kennedy involved equations derived by Valle (*Annali Geofis.*, **5,** 41; 1952). Strictly, these equations apply to a solid, but Valle supposed that at very high pressures a liquid behaves sufficiently like a solid for the same theory to be applicable. At the same time, however, he supposed that the core behaves like a liquid in that the shear wave velocity is zero. The assumptions are thus physically incompatible—a defect which, Frazer claims, invalidates the theory's application to the core unless some more convincing justification can be given. Similar claims are made against the second method (involving Gruneisen's parameter Γ) used by Higgins and Kennedy.

But if Frazer's refutation of the case against convection put forward by Higgins and Kennedy is valid, it cannot necessarily be taken that convection actually occurs. Even if the simplifying assumptions adopted by Higgins and Kennedy are avoided, integration of the more general equation for the adiabatic gradient is still critically dependent upon obtaining accurate values of the core's coefficient of thermal expansion (α) and specific heat at constant pressure (C_p). In fact, accurate values of these parameters are not known. It turns out that comparatively small changes in the α/C_p ratio which are well within the present limits of uncertainty are sufficient to place the adiabatic temperature curve well above or well below the melting temperature curve. According to Frazer, it is thus not possible to say whether convection in the core is feasible or not. To that extent the convection implied by the existence of the geomagnetic field remains but an article of faith.

Heterogeneous nuclear and messenger RNA

from our
Molecular Genetics Correspondent

ONE of the most important characteristics of messenger RNA synthesis in eukaryotic cells is the processing system in the nucleus which cleaves the mature messenger molecule from its much larger precursor. Because only a small proportion of the heterogeneous nuclear RNA (HnRNA) comprising the precursor is utilised to produce messengers—most of it is rapidly degraded within the nucleus—the maturation process represents a critical step in gene expression in eukaryotes. Three articles in the first issue of *Cell* are devoted to intriguing questions raised by the relation of HnRNA and mRNA.

A vital step in processing the large HnRNA to the much (up to ten times) smaller mRNA is the addition of a sequence of polyadenylic acid, probably one base at a time, to the 3′ end of the HnRNA molecule. This is then cleaved to generate the poly(A)-containing mRNA. In eukaryotic nuclei, the length of this poly(A) is usually about 200 bases and inhibition of its addition prevents messenger production.

What is the function of the poly(A) sequence? Is it related solely to the maturation process, perhaps because it is needed for nucleocytoplasmic transport of mRNA, or does it serve some function in the cytoplasm? That its role is not solely nuclear has been inferred from experiments which demonstrated the presence of poly(A) in viral RNAs which function solely in the cytoplasm. Support for the view that poly(A) plays some part essential to the function of all mRNAs in eukaryotic cells is provided also by the observation that the messengers of mitochondria possess a short length of poly(A).

Mitochondria possess a protein synthetic system which differs in several ways from that of the surrounding cytoplasm, in some ways showing analogies to bacterial protein synthesis. The presence of poly(A) sequences has been used to isolate a mitochondrial RNA fraction which seems to represent the messengers; their length of poly(A) is fifty-six bases, somewhat shorter than that of cytoplasmic mRNA. An observation now reported by Hirsch *et al.* (*Cell*, **1**, 31; 1974) is that the size of the poly(A) fragment is virtually identical in human and insect mitochondria. Although no function has yet been suggested for the poly(A) in mitochondrial mRNA, its presence is clearly unrelated to transport and may be solely intramitochondrial, perhaps related to whatever function it has in the cytoplasmic messengers. Also of importance is the observation that the poly(A)-containing mitochondrial mRNA can be fractionated into some eight species, which together with the tRNA and rRNA sequences previously identified may account for transcription of the complete mitochondrial genome. These experiments suggest a view of the mitochondrion as an entity whose evolution has to some extent been independent of the surrounding cytoplasm.

Extending their previous studies to report now the first determination of the location of specific sequences within the non-messenger parts of nuclear HnRNA, Molloy, Jelinek, Salditt and Darnell (*Cell*, **1**, 43; 1974) suggest a tentative structure for the large precursor. That the sequence destined to become mRNA lies at the 3′ end of the molecule to which poly(A) is added is reasonably well established. But a crucial question concerns the composition of the remainder of the molecule, which is to be degraded. It is especially important to identify the junction between the mRNA and non-mRNA regions and to establish the criteria used by the maturation system for selecting the messenger.

The technique used by Molley *et al.* was to isolate poly(A)-containing HnRNA on poly(U) sepharose columns, break these molecules with alkali, and then reisolate the 3′ terminal sequences (which still contain poly(A)). By examining the properties of fragments of different lengths, it is possible to deduce the nature of the sequences located on the 5′ side of the messenger part of the molecule.

Because HnRNA can hybridise to DNA at rates more rapid than mRNA, it appears to contain transcripts of repetitive sequences absent from the messenger sequences. Using fragments isolated by the alkaline cleavage of HnRNA, Molloy *et al.* found that the first 3,000–4,000 3′ nucleotides hybridise at the same rate as mRNA, but that as the fragment length increases to 8,000 nucleotides the rate increases 3–4 times to the value characteristic of HnRNA. Since mRNA is in general about 3,000 nucleotides long, this result implies that the HnRNA molecule must contain repetitive sequences fairly near to the 5′ end of the mRNA section. Because the rate of hybridisation remains constant from a fragment length of 8,000–30,000 bases, the rapidly hybridising regions in this part of the HnRNA may be about equally interspersed with less rapidly hybridising regions.

Another marker used to pin down specific parts of HnRNA is the presence of oligo(U) fragments, generally about thirty bases long. The poly(A) terminated HnRNA molecules larger than some 20,000 nucleotides possess about three oligo(U) fragments each; shorter HnRNAs possess a smaller proportion of these sequences; and mRNA lacks them altogether. More than 90% of the oligo(U) segments are located further than 12,000 nucleotides from the 3′ terminus and may be clustered at closely located sites.

A generalised structure for HnRNA thus locates mRNA and poly(A), occupying about 3,000 nucleotides, at the 3′ end. Pointing to the observation that rapid hybridisation of HnRNA depends on both cleavage and denaturation, Molloy *et al.* suggest that hairpin structures, in general less than 500 bases in length, may occupy the sequences up to about 15,000 nucleotides from the 3′ terminus. Sequences of oligo(U) may then be located towards the 5′ end of the HnRNA.

Whereas the addition of poly(A) seems to be a feature distinguishing the mRNA sequences of HnRNA which are to be conserved, a different mechanism has been known for some time to apply to ribosome RNA in eukaryotes. A large precursor, sedimenting at 45S in mammalian cells, is methylated only in certain regions; and these regions ultimately become the 18S and 28S rRNAs. The rest of the molecule is degraded. Methylation has until now been thought to be a prerogative of rRNA and tRNA (in which it is necessary for the functions of the molecule) alone. But Perry and Kelley (*Cell*, **1**, 37; 1974) now provide evidence that mRNA of mouse L cells is also methylated. After labelling with ^{3}H-methionine and ^{14}C-uridine, the ^{3}H/^{14}C ratio of the poly(A)-containing RNA of the polysomes is about one fifth of that of rRNA. From this comparison, Perry and Kelley calculate that the proportion of methyl groups in mRNA is about 0.22%. This means that an average mRNA of 3,000 nucleotides should bear six to seven methyl groups.

Both base and ribose moieties suffer methylation, the proportion of base methylation in mRNA being greater than in rRNA. A preliminary analysis suggested that there may be only one

Solar neutrino problem still unsolved

A Few months ago, Prentice suggested that the absence of neutrinos from the Sun could be explained if that star has a hydrogen-free core amounting to about 0.02 or 0.03 $M_\odot$ (*Mon. Not. R. astr. Soc.*, **163**, 331; 1973). Such helium inhomogeneities reduced the neutrino flux by a factor of 10, or least for the simple polytropic models used by Prentice. But now more detailed calculations by Demarque *et al.* (*Mon. Not. R. astr. Soc.*, **165**, 19P; 1973) suggest that this is not the case for realistic solar models.

These detailed models confirm Prentice's qualitative discovery, and show that both central temperature and ^{8}B neutrino flux are reduced, with a minimum for a core mass of 0.025 $M_\odot$; however, this minimum is only 20% less than the 'normal' flux of ^{8}B neutrinos. Even so the results for idealised static models are still better than those obtained when an evolutionary sequence is calculated. A model evolved from a zero age situation in which the helium core was 0.025 $M_\odot$ reached one solar luminosity after 4.7 × 10^{9} yr, when the ^{8}B neutrino flux was only 1.5% below that for a 'standard' Sun evolved from a homogeneous zero age state.

or a few species of methylated base in the messenger. Some data also suggest that the methylated bases may be concentrated at adjacent nucleotides, perhaps located towards the 5′ end of the molecule; and Perry and Kelley suggest that the methylation sites are probably located in non-coding sequences of the messenger.

The proportion of methylated bases in HnRNA is much lower than in mRNA, being barely detectable. It seems possible therefore that methylation may take place in the HnRNA precursor only in those sequences destined to become mRNA, a situation analogous to the maturation of ribosomal RNA. What part this methylation plays in messenger selection must of course at present be a matter for speculation, but it is tempting to suppose that it may be involved in the selection of messenger sequences for conservation and transport to the cytoplasm.

The rationale for the evolution of the complex processing systems for both nuclear mRNA and rRNA remains elusive; it is difficult to see why half of the rRNA precursor and an even greater proportion of the HnRNA precursor should be synthesised only to be degraded. That the maturation process may be part of a system for gene control—rather than simply a feature of the mechanism of mRNA production—remains an intriguing possibility. But the presence of poly(A) in mitochondrial mRNAs throws a puzzling light on its relationship to this process, although methylation now also becomes a candidate for the role of ensuring specificity of selection. Although premature at present, it is tempting also to conclude that a general structure may be valid for all HnRNA molecules so that its features too may be important in regulating gene expression in higher cells.

Controlling toxicity of chromate waste heaps

from our Plant Ecology Correspondent

REVEGETATION of toxic spoil from industrial processes can be achieved only when the true nature of its toxicity has been established. This fact was ably demonstrated by Breeze's preliminary work on the chromate waste heaps of the Croal Valley in Lancashire (*J. appl. Ecol.*, **10**, 513; 1973). Gemmel (*Environ. Poll.*, **5**, 181; 1973) has now found that the interaction of various ions in the chemically diverse waste material of these chromate spoil heaps necessarily complicates any model of toxicity in this system.

Gemmel's technique involved the collection of samples from the waste heaps, analysing them chemically, diluting them with lime-free sand and testing their toxicity biologically using white mustard (*Sinapis alba*) as a test plant. In chemical analysis he found that levels of chromate, calcium, sulphate, hydroxide and bicarbonate ions were all high enough in the unweathered material to be possible causes of toxicity; in weathered waste magnesium ions also reached high concentrations.

Alleviation of toxicity before revegetation requires the determination of which of these ions is most important in the inhibition of plant growth. Gemmel attempted this by growing mustard on samples of known chemical composition and analysing the data so produced by means of correlation matrices linking dry matter production with the levels of the ions of potential interest. Highest toxicity was correlated with chromate and bicarbonate. Magnesium and calcium ions were positively correlated with dry matter production, indicating that these may alleviate the effects of the toxic ions. This is of particular interest since calcium is known to reduce the toxicity of heavy metal cations, such as lead (Wilkins, *Nature*, **180**, 37; 1957).

If, as seems likely, toxicity is broadly determined by chromate but is modified by other ions at any given chromate level, analysis of data by simple correlation is inadequate. Gemmel tries to overcome this by grouping his samples on the basis of their chromate content together with other criteria and subsequently submitting the groups to multiple discriminant analysis to examine their validity. He then produces correlation matrices for ions and dry matter production of mustard within the groups. These indicate that hydroxide ions exert a synergistically toxic effect at medium and high chromate concentrations.

This approach, however, does not overcome the disadvantages of relying on correlations in what is evidently a chemically complex situation; for example, positive correlations between certain ions, such as magnesium and bicarbonate in weathered waste, may result from a common origin in the spoil, in this case magnesium hydroxide, reacting with carbon dioxide. If one of these ions should exert an influence on and therefore be correlated with plant growth, the other is bound to show a similar effect in terms of correlation.

As in all such multivariate situations, the ultimate solution lies in an experimental design which will allow the manipulation of the concentrations of individual ions while other ions deemed critical can be held constant. Without such an approach knowledge of the key factors controlling toxicity in waste materials must continue to be speculative.

Rhythms and the Earth's rotation

from a Correspondent

TIME is simply a progression of events, so that different clocks measure different things and their comparison gives a vital synthesis to all the sciences, even if the philosophical puzzle of the real nature of time still remains. A conference organised by G. D. Rosenberg and S. K. Runcorn on January 8–10, in the School of Physics of the University of Newcastle upon Tyne, on biological clocks and the rotation of the Earth, considered comparisons between atomic, astronomical and biological clocks and the ways in which these give information, not only on the changes in the length of the year and lunar month, but on basic organic processes, the evolution of the Solar System, changes in physical 'constants', and so on.

Several speakers (G. R. Clark, University of New Mexico; J. W. Evans, Memorial University of Newfoundland; C. Hall, University of California, Los Angles; Rosenberg; and M. A. Whyte, University of Hull) were concerned with recent experiments to determine when organisms, particularly marine bivalves and corals (R. W. Buddemeier, University of Hawaii) actually put on growth increments. It seems strange that such vital aspects have been so little studied as it is of obvious importance that some organisms show daily increments and others show additional tidal, seasonal, reproductive and circadian growth rhythms. Different animals were discussed in which one or more of these patterns could be determined, mostly by optical means, although it was sometimes necessary to resort to staining or X-ray analyses. But I. Thompson (Princeton University) showed that even when isolated from all obvious environmental stimuli, including food, some bivalves seem to regulate their biological clock with the movements of the Moon, presumably through changes in the gravity field or electromagnetic changes, so that marine organisms well below the tidal and even the photic zones may still show externally related growth rhythms.

Such fundamental findings have already become important to palaeontologists, because observations of past growth bands can determine past environments, not only on a gross scale, by the palaeolatitudinal implications of either seasonal banding or the thickness of the incrementals, but also in terms of past water depths, compositions of the substrate and local ecological relationships. The greatest interest, however, was in new results obtained by W. B. N. Berry (University of California) and G. A. L. Johnson (University of Durham) from counts of different types of increment shown by a variety of fossils several 100 million years old which have enabled the number of days in the month and in the year to be determined. Calculations from stromatolites more than 2,000 million years old have also been made by G. Pannella (University of Puerto Rico) and R. E. Mohr (University of Minnesota. It turned out that many counts have, in fact, been made, but only a daring few had, as yet, been published, mainly from an uncertainty of either their meaning or reliability. Yet there was remarkable agreement that these counts showed that Earth had spun faster in the past and that its run-down has not been constant, but decelerated very slowly through most of the Precambrian and much of the Phanerozoic. Alterations in the rate of deceleration possibly correlate with geological or geophysical changes.

The problems of counting how many lines actually exist, or seem to exist, between yet another series of bands of varying distinctness, was in stark contrast to the precision of astronomical timing. Since 1955 the atomic clock has enabled incredibly accurate measurements of the rate of the Earth's rotation to be made and has already confirmed the somewhat less precise measurements described by N. P. J. O'Hara (Royal Greenwich Observatory) on the observations of the changing positions of the planets against the stars. L. V. Morrison (Royal Greenwich Observatory) demonstrated how these results show a secular deceleration of the Earth's rotation during the past 250 years of 42 ± 6 seconds of arc century^{-2} (2 ms century^{-2} of time). Superimposed on this are irregular fluctuations which were attributed to wind patterns in the short term and to changing electromagnetic coupling between the Earth's core and mantle for longer periods—although doubts were expressed as to whether this force was really adequate.

F. R. Stephenson and P. M. Muller (University of Newcastle upon Tyne) presented results culled from mediaeval chronicles, the classics, Babylonian and ancient Chinese manuscripts and showed that total eclipses during the past 2,500 years indicated a uniform secular deceleration of about 83 arc s century^{-2} for the Earth and 32 arc s century^{-2} for the longitudinal position of the Moon. Such rates extended over geological time, however, would result in the collision of the Earth and Moon only 600 million years ago, which is inconsistent with the geological evidence. K. M. Creer (University of Edinburgh), J. A. Jacob (University of Alberta) and Runcorn, with J. Gribbin (*Nature*), J. Keeney (Superior Oil), D. H. Tarling (University of Newcastle upon Tyne), D. H. Weinstein (Superior Oil) and P. Wesson (University of Cambridge) all offered possible explanations of varying credibility for the variation in the slow-down of the Earth, which was attributed to a lack of deep oceans in the past, changes in the atmosphere or geomagnetic field, to changes in either the gravitational or Hubble constants (G and H). The final conclusion was that biologists should try to find which organisms best represent which rhythm and why, so that palaeontologists can then determine which fossils to study to enable geologists and geophysicists to eliminate at least some of their variables.

Membrane components in virus infected cells

from a Correspondent

How do cell membranes assemble? This question is obviously unanswerable at present, but the meeting of the Cell Surface Discussion group that took place on December 11 at the University of Warwick was centred on a model system that may well provide important evidence leading to a correct solution of this problem. The meeting, organised by Professor D. C. Burke, was concerned with biosynthesis of membrane components in virus infected cells.

Participants at the meeting, by no means all virologists, were introduced by R. W. Compans (Rockefeller University, New York) to the many attractions of the viral system. The final step in enveloped virus maturation (for example, simian virus SV5) involves budding of nucleocapsids through modified regions of the surface membrane of the permissive host cell, modified, that is, by the insertion into the membrane of several viral gene products. These products include polypeptides which are glycosylated by host cell glycosyl transferases and form the glycoprotein species of the envelope of fully assembled virions. During virus replication, the synthesis of host cell proteins is often severely limited or shut off altogether. Development of surface membrane 'patches' of relatively simple composition—in most cases one or two virally coded glycoproteins—can therefore be studied in infected cells. The patches can be identified by specific antibodies directed against the virus. Using antibodies tagged with ferritin, very clear areas of the host cell membrane are visualised in this way.

Insertion of the virally specified antigens into the plasma membrane of the host cell many occur in a single step by fusion with the plasma membrane of an intracellular vesicle containing viral glycoproteins. Alternatively, insertion of the first viral antigen into the host cell membrane forms a nucleation point for the insertion of other neighbourly viral antigens. Yet another possibility is that insertion is random all over the host cell surface and redistribution takes place in a later step in viral maturation. The latter hypothesis invokes the by now familiar principle of membrane fluidity and migration of large molecules in the plane of membrane structures.

Membrane fluidity of lectin-binding sites in cells infected with budding viruses was discussed by P. Reeve (University College Hospital Medical School, London) and H. Becht (Justus-Liebig University, Giessen). Concanavalin A and wheat germ agglutinin (WGA) agglutinate cells infected with certain orthomyxoviruses and paramyxoviruses, and block virus release from these cells. Since infected cells bind no more radiolabelled concanavalin A molecules than do uninfected cells that do not agglutinate, agglutination is believed as in other systems to result somehow from clustering of surface membrane glycoproteins and is not due to the exposure of new lectin-binding sites in infected cells, at the budding sites for instance. Concanavalin A also agglutinates viruses such as fowl plague but not protease-treated virions from which the surface carbohydrate has been removed. In the latter case, however, receptor sites are now exposed for binding to *Dolichos biflorus* lectin. Becht suggested that these sites are present in glycolipids containing N-acetyl galactosamine.

If membrane fluidity and redistribution of viral glycoproteins turns out to be the correct mechanism by which viral specific patches form in the surface membrane of infected host cells, the way in which the redistribution is directed in viral maturation remains unclear. Association of viral proteins molecules (matrix M protein) on the cytoplasmic side of membrane patches containing viral antigens was described for SV5 by R. Cartwright (Animal Virus Research Institute, Pirbright), for VSV and for fowl plague virus by J. Skehel (National Institute for Medical Research, Mill Hill). These molecules were not seen associating with other areas of the host cell membrane containing no viral envelope antigens. It is possible that it is this association which induces formation of the 'patches' rather like the interaction of spectrin and the major erythrocyte glycoprotein. Compans, however, reviewed the evidence that in many viruses the envelope glycoproteins do not penetrate the lipid bilayers to any great extent and certainly do not span it. If this is true also for the 'patches', the question that then arises is what is the recognition mechanism by which viral core proteins station themselves immediately on the cytoplasmic side of areas of the host cell membrane containing externally-oriented viral glycoproteins? The specificity presumably must lie in the lipid composition of these membrane patches.

Skehel reported that during infection of chick cells with fowl plague there is a build up of viral glycoproteins in the host cell plasma membrane. Insertion of large amounts of these molecules, the viral haemagglutinin and neuraminidase, into the membrane is not compensated for by a loss of host cell membrane components: all of the host cell polypeptides can be identified in apparently normal amounts in the plasma membrane fraction of virally infected cells. Again, however, as in the SV5 system the virally coded glycoproteins form discrete clusters. This was shown after sonication of plasma membrane preparations of infected cells to form vesicles. A vesicular subfraction absorbed preferentially to red blood cells and contained in addition to the expected viral haemagglutinin, the matrix protein and neuraminidase. Moreover, some host cell membrane proteins were present. By contrast, an unabsorbed subfraction contained in addition to host cell membrane proteins, the viral neuraminidase and the uncleaved haemagglutinin.

The mechanism of the cleavage process of viral envelope precursor polypeptides received considerable discussion. Perhaps the most thoroughly studied system is in cells infected with Semliki Forest virus. S. I. T. Kennedy (University of Warwick) summarised the work of the Warwick group and of other workers on the replication of Semliki Forest virus, an alphavirus, in BHK cells. When infected cells are treated with protease inhibitors (TPCK), several viral gene products accumulate intracellularly. The largest of these is a polypeptide of molecular weight 160,000, a value that is a little short of the translational product expected of the major (26S) RNA present in virally infected cells. The polypeptide normally undergoes a series of proteolytic cleavages to produce first a polypeptide of molecular weight 120,000 and finally a core protein (molecular weight 35,000) and two envelope glycoproteins EP1 and EP2 (molecular weights 57,000 and 54,000). Intermediate partially cleaved polypeptides have also been identified. Pactamycin 'blocking' experiments indicate that the core polypeptide is N-terminal to the envelope polypeptides EP1.EP2. A small carbohydrate rich fragment is apparently inserted between EP1 and EP2 in the uncleaved precursor and is cut out during the fashioning by limited proteolysis.

R. Weiss (Imperial Cancer Research Fund, London) argued for the presence of RNA tumour virus antigens on some apparently normal chick embryo cells. Cells (GS$^+$) genetically endowed with the ability to synthesise this surface antigen, a glycoprotein support the production of infective virus (Bryan strain of RSV) whereas negative (GS$^-$) chick cells do not. The antigen is incorporated into released virus and is necessary for subsequent attachment of virus to new host cells. Two other products studied by Weiss are coded by host genes; one of these is a surface receptor recognised by the GS$^+$ product. The other host gene specifies a surface protein product that prevents infection by blocking the receptor site when this is present. Attempts to expose the receptor in blocked cells by mild proteolysis have been unsuccessful. The inhibitor gene segregates with the *GS*$^+$ gene and it is possible that the two functions represent pleiotropic expressions of the same gene.

Cytochemical approach to hormone assay

from a Correspondent

THE main conclusion of the symposium on the cytochemical approach to hormone bioassay, held at the Kennedy Institute of Rheumatology in London on December 6, is that quantitative cytochemistry is now so precise that it can be used to measure the response produced by as little as 50 fg ml^{-1} of a hormone acting on its target cells. Consequently even 0.1 ml of plasma is sufficient for measuring the content of corticotrophin in the blood of the patient or animal; the plasma sample is measured at two dilutions (1:100 and 1:1000) and the results should agree to ±15%.

Cytochemical methods for measuring the effect of four hormones are now in use. The first method, for the bioassay of corticotrophin (ACTH), is the most fully developed and the whole morning was devoted to the technique and its applications. The first part of the afternoon was concerned with the development of this method to the bioassay of luteinising hormone (LH) and with preliminary results concerning the levels of this hormone during the menstrual cycle. The second part of the afternoon was devoted to two of the more recent investigations—studies on the effect of thyroid stimulating hormone (TSH) and the long-acting thyroid stimulator (LATS)—and those which may lead to a new assay for gastrin.

Essentially the methods for all these assay studies are similar. The target tissue is removed from the animal and cut into segments. Each segment of tissue is maintained for 5 h in non-proliferative maintenance culture. This manoeuvre removes the target cells from the hormone influence of the animal and allows them to recover from the trauma of excision. The culture medium is then removed and replaced for a matter of only minutes with culture medium containing either graded concentrations of the standard hormone preparation (to obtain a response: concentration calibration graph) or one of two dilutions of the unknown plasma. The tissue is chilled, sectioned and reacted by a cytochemical method for the most appropriate biochemical activity affected by the hormone. Much of the sensitivity of these assays depends on the use of scanning and integrating microdensitometry for measuring this response solely in the target cells; moreover, they are within-animal assays so eliminating the notorious biological variability of most bioassays. The speed with which at least one of these assays can be performed has been increased remarkably by a new modification in which 20 μm sections are used instead of segments of the target tissue.

The past ten years have seen a revolution in endocrinology caused by the advent of radioimmunoassay of hormones. This technique, however, has two drawbacks. In the case of certain hormones, it is very difficult to measure low physiological, or subnormal, concentrations by such methods and they sometimes detect immunopotent molecules which may have little or no biological activity. The advantages of the cytochemical bioassays are that they easily encompass the measurement of such subnormal levels and, of course, measure only biologically active molecules. In the case of ACTH the divergence of the radioimmunoassay and bioassay results became marked only during the rapid rise and fall which occurred during the hypoglycaemia stress tests, done to determine how well the hypothalamo–pituitary–adrenal function was maintained in rheumatoid patients treated with ACTH or with corticosteroids. A more striking divergence was seen in the levels of LH; this, if fully established, could have important implications in the present understanding of ovulation.

The techniques were first developed in the Division of Cellular Biology at the Kennedy Institute in collaboration with the Department of Chemical Pathology of the Charing Cross Hospital Medical School. They were then extended by the Department of Chemical Pathology at St Bartholomews Hospital Medical College and are currently also in use in the Department of Pharmacology, the Royal Free Hospital Medical School, and in the Department of Medicine at Newcastle.

A case for regional culture collections of microorganisms

S. M. Martin

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Dr Martin describes the proposals for regional culture collections of microorganisms especially in developing countries.

UNTIL fairly recently, little attention has been paid to the diminution of the genetic pools of higher plants and animals. On the other hand, since the earliest days of their science, microbiologists have been forced to conserve the genetic resources of their microbes, albeit they have not always expressed their concern in terms of preservation of genetic pools. Microbiologists, of necessity, have had to preserve their cultures in a viable and physiologically unaltered state since relatively little can be learned from dead microorganisms. As a consequence, a number of very fine culture collections have been established and considerable research effort has been expended on developing techniques suitable for the long-term storage of living microbes.

In 1962 a meeting of culture collection specialists (266 scientists from twenty-six countries) was held in Ottawa, Canada. Recognising the vital role of culture collections in the science and technology of microbiology, the delegates recommended that International Association of Microbiological Societies (IAMS) establish a section on culture collections. IAMS acceded to this request at its next meeting and the first formal meeting of the Section (later to become the World Federation for Culture Collections [WFCC]) took place in Paris in 1966. In 1968 an international conference on culture collections took place in Tokyo. In attendance were 300 scientists from fifty-two countries, twenty-seven of which could be classed as developing. A third conference was held in July 1973 in Sao Paulo, Brazil.

Parallel to these developments, the Japanese Federation of Culture Collections in 1963 submitted, through the Japanese delegation to Unesco, a proposal for a 10-yr programme for research in microbiology. Heavy emphasis was placed on the need for an international federation of culture collections and for the establishment of specialised culture collections throughout the world. The proposal was referred to IAMS and in 1964 IAMS presented its brief to Unesco. Unesco initiated action by convening a meeting of culture collection specialists in Paris in 1966.

Brief to UN

Over the past decade, WFCC has given a great deal of consideration to problems faced by culture collections and has initiated a number of relevant programmes. Thus, it was that WFCC found itself in an excellent position to submit to the secretariat of the UN Conference of the Human Environment (Stockholm 1972) a brief on the conservation of genetic pools of microorganisms. The main points made in the brief were: (1) In any consideration of the conservation of the genetic resources of the world, it is imperative that the need to conserve the genetic pools of microorganisms be recognised. (2) Man, and other forms of life, could not exist without microorganisms since it is through their diverse activities that the essential elements for nature are recycled. Without this recycling, all life forms would soon die. (3) Although the greatest amount of microbial activity goes on in nature without the guiding hand of man, man does apply microorganisms in a great many ways for his own betterment. (4) Wherever and whenever microorganisms are applied scientifically for man's benefit, it is essential to have available a large number of diverse sources of microorganisms. (5) Culture collections are 'libraries' or 'museums' of living microorganisms, where cultures are received, maintained in a viable and genetically unaltered state and studied, and from which cultures are dispatched to other workers. (6) Culture collections also act as clearing houses for information concerning the cultures they maintain. (7) Culture collections quite often provide identification of newly isolated strains of microorganisms.

As a result of the WFCC and other similar briefs, a series of recommendations dealing with the conservation of the genetic resources of microorganisms came out of the Conference.

To some it was difficult to see how the UN would organise the proposed coordination of collections of microorganisms. To others, it was difficult to understand why the conservation of microbial gene pools should be of concern when there already exist a number of fine collections of cultures of microorganisms. "It is unrealistic to establish culture collections in developing countries" is a statement one hears voiced rather frequently. Such a statement can be justified only if by it one means that it is unrealistic to maintain a large service collection, such as the American Type Culture Collection (ATCC) or the National Collection of Type Cultures (NCTC), in every developing country in the world. To accept the statement at its face value would be to take the position that it is unrealistic to carry on work with microorganisms in developing countries.

What then is a culture collection? It is precisely what the term implies: a collection of cultures of living microorganisms, with no constraints as to number of strains or types of microorganisms held. Such a broad definition is necessitated by the fact that the same principles apply to the conservation of a single strain as to the conservation of many. Furthermore, the preservation of microorganisms in a healthy and physiologically unaltered state is, or should be, of equal concern to the worker who uses only a few strains as it is to the curator of a large service collection. With this basic definition in mind, we can then logically recognise several types of culture collection ranging from the large service collection, such as ATCC or NCTC, whose prime function is the supply of cultures on demand, to the small private collection which may or may not distribute its cultures. Between these extremes lie a host of intermediates.

Plan of action

In August 1972, WFCC submitted to the conference secretariat an 'Action Plan' for the implementation of the recommendations made at Stockholm. The following is an interlock of the pertinent recommendations and the WFCC proposals:

> "It is recommended that governments agree to an international programme to preserve the world's genetic resources.

- Active participation at the national and international levels is involved.
- An international network is required with appropriate machinery to facilitate the interchange of information and genetic material among countries.
- Both static (seed banks, culture collections, etc.) and dynamic (conservation of populations in evolving natural environments) ways are needed.
- Action is necessary in several inter-related areas:
  (1) survey of genetic resources
  (2) inventory of collections
  (3) exploration and collecting
  (4) conservation
- Although the international programme relates to all types of genetic resources, the action required for each resource will vary according to existing needs and activities."

WFCC is fully prepared to serve as the microbiological partner in the international programme to preserve the world genetic resources.

> "Compile or extend, as necessary, registers of existing collections.•
>
> - In respect to microorganisms, it is recommended that each nation develop comprehensive inventories of culture collections:
>   (1) a cataloguing of the large and small collections and the value of their holdings is required, rather than a listing of individual strains
>   (2) many very small but unique collections, sometimes the works of a single specialist, are lost
>   (3) governments should assure that valuable gene pools held by individuals or small institutes are also held in national or regional collection."

WFCC has already prepared an extensive documentation on large and small collections in all parts of the world and their holdings. WFCC proposes to expand and continually update this information and to make it available to governments and international, regional and national organisations.

> "Microorganism germ plasm: cooperatively establish and properly fund a few large regional collections.
>
> - Full use should be made of major collections now in existence.
> - To provide geographic distribution and access to the developing nations, regional centres should be established in Africa, Asia and Latin America and the existing centres in the developed world should be strengthened."

In conjunction with IAMS and the International Cell Research Organisation/Unesco Panel for Microbiology and in consultation with other UN agencies, WFCC proposes setting up a working party to be charged with the following: (1) Choosing suitable sites for regional culture collections in Africa, Asia and Latin America. (2) Providing guidelines for the establishment of these regional collections. (3) Establishing mechanisms for ensuring that continued quality and functioning of these collections is maintained.

> "Collaborate to establish a global network of national and regional institutes based on agreements on the availability of material and information, on methods, on technical standards, and on the need for technical and financial assistance wherever required.
>
> - Such cooperation would apply to all types mentioned above.
> - Standardised storage and retrieval facilities for the exchange of information and genetic material should be developed:
>   (1) information should be made generally available and its exchange facilitated through agreement on methods and technical standards
>   (2) international standards and regulations for the shipment of materials should be agreed upon
>   (3) basic collections and data banks should be replicated in at least two distinct sites, and should remain a national responsibility
>   (4) standardised and computerised system of documentation is required.
> - Technical and financial assistance should be provided where required:
>   (1) areas of genetic diversity are most frequently located in those countries most poorly equipped to institute the necessary programmes."

Based on its extensive experience in the field of the distribution of cultures and of information pertaining thereto, WFCC considers itself competent to contribute to the standardisation of storage and retrieval facilities for the exchange of information and microbial genetic material. The necessary framework is already set up. WFCC could contribute, through its committee on documentation, to the establishment of "a standardized and computerized system of documentation". The information already collected, and to be collected in the future, is readily available to all.

WFCC has set up a working group to study "international standards and regulations for the shipment of materials".

Regional centres

The maintenance of data and important cultures in more than one centre is one of the guiding principles of WFCC.

> "The need for liaison among the parties participating in the global system of genetic resources conservation requires certain institutional innovations.
>
> - It is recommended that the appropriate United Nations agency initiate the required programme on microorganism germ plasma
> - periodic international conferences involving those concerned with the maintenance and research on gene pools of microorganisms should be supported
>   (1) such a programme might interact with the proposed regional culture centres by
>   (*a*) assuring that each centre place high priority on the training of scientists and technicians from the developing nations
>   (*b*) acting as a necessary liaison
>   (*c*) lending financial assistance to those collections established outside the developed countries
>   (2) the international exchange of pure cultures of microorganisms between the major collections of the world has operated for many years and requires little re-enforcement
>   (3) study should be particularly conducted on waste disposal and recycling, controlling diseases and pests, and food technology and nutrition.

This recommendation relates directly to the existing programme of conferences and training courses of WFCC. The WFCC is prepared to undertake expansion of this programme to ensure interaction with the proposed regional culture collection centres.

It is worth noting at this point that the concern for culture collections in developing countries has not been exclusive to the WFCC and curators of culture collections. In the *Report and Proceedings of the Expert Group Meeting on the Manufacture of Chemicals by Fermentation*, Vienna, 1969 (United Nations Industrial Development Organisation publication E.71.II.B.6) it was recommended that

> "A few regional culture collections should be established, which would supply cultures and transmit information and technical expertise relating to cultures. These should be located in the vicinity of fermentation plants or universities doing applied fermentation research. Staff members should be available capable of solving microbiological problems in the plants of their regions. These centres might even supply inoculum to plants of their regions. They would also be good places to train tech-

> nicians in production control and techniques (determination of *p*H, product stability, etc.). Training would last up to one year. Probably centres would be needed currently in Central Africa, India, the Middle East, North Africa and South America."

Unfortunately it seems that UNIDO failed to act on this recommendation.

Developing countries

It is most reasonable to suggest that major sources of microorganisms, the large service collections, should be limited to relatively few well equipped and well supported institutions strategically placed throughout the world. (At this point it is perhaps well to re-emphasise that culture collections do much more than simply distribute cultures; they also act as important identification, information and training centres.) Unfortunately, the large service collections are located almost exclusively in the highly industralised nations. For reasons of either cost or distance, workers in developing countries are frequently denied these goods and services. Furthermore, it must be recognised that, although service collections operate on an international basis, they are in fact set up to meet primarily national needs. Indeed the very name of the collection usually carries some designation to the effect that it is a national collection. Developing countries have their own peculiar problems and there is need for service collections which specialise in those organisms and services having relevance to these areas.

WFCC feels that the proposed regional culture collections should concentrate on the maintenance of strains of microorganisms which are related to problems of the region. Furthermore, regional collections, wherever possible, should be located in existing research centres concerned with work relevant to the cultures maintained and they themselves should be focal points for research and training. One can envisage a regional collection as being composed of several specialised sub-collections each located in a different institution but functioning as a unit.

Personnel of the proposed regional centres must be well trained in all aspects of the organisation and operation of culture collections. Senior scientific personnel should have the opportunity to spend approximately a year working in one or two major collections in the developed world before the centres actually become operational. Technical personnel should attend at least a 2-3 week group training course in culture preservation. Once the regional centres are fully operational, they should act as centres for training microbiologists of the region. As regards the importance of the proposed regional culture collections as training centres, it should be emphasized that the conservation of microorganisms is properly as much a matter of concern for the microbial biochemist or the fermentation technologist who uses only one or two organisms as it is for the curator of a large service collection.

It is obvious that any consideration of culture collections should be made in the overall concept of microbiology. The collection staff should consider itself part of a regional 'microbiology team' concerned with the application of the activities of microorganisms to the fulfillment of human social and economic needs. Thus the centres can make a significant contribution to upgrading all aspects of microbiology in developing countries.

In summary, it does seem reasonable that the UN initiate the formation of regional culture collections in the third world, since the need exists and the process could be carried forward in a rapid and logical fashion.

Tectonic evolution of continents in early Proterozoic times

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In early Proterozoic times, complex systems of mobile belts were developed within large continental masses as a result of the internal distortion of these masses which accompanied bodily movement relative to the poles. The arrangement of structures formed by these processes differs from that characteristic of both Archaean and Phanerozoic tectonic regimes.

THE portions of the continental crust which acquired their tectonic patterns in early Proterozoic times show a structural style which differentiates them on the one hand from Archaean provinces whose structures record widespread mobility and on the other from late Proterozoic and Phanerozoic provinces whose structures record the disruption and collision of rigid plates. Palaeomagnetic evidence[1,2] suggests that over the period from about 2,500 to 1,300 Myr ago crustal masses of continental dimensions were moving as units relative to the poles[3]. The arrangement of certain major structural features also points to the existence at this period of large sheets of continental crust[4,5]. There is, however, abundant evidence that these sheets did not behave like the rigid lithosphere plates of the present day: a glance at the relevant tectonic maps shows that they were mosaics in which more stable massifs were enclosed by more mobile belts. Although early Proterozoic plates moved as coherent entities, they were not rigid and suffered extensive internal deformation and metamorphism while in transit. We propose to examine here the structural patterns which resulted from this distinctive style of tectonics.

North America

The massifs which remained relatively stable over the period 2,500–1,700 Myr—the Superior, Slave and eastern Nain provinces—are set in a network of mobile belts which includes the Churchill province and its extensions (Fig. 1). Marginal structures comparable with those developed at younger destructive plate boundaries mark the Labrador and Coronation geosynclines where Proterozoic fold belts incorporating eugeosynclinal assemblages flank the stable massifs[7,8]. Elsewhere, however, the marginal structures of the massifs are of a different type. The majority are defined by tectonic lineaments belonging to a widely developed north-easterly set, locally marked by lines of intrusions, by mineralised zones or by narrow tracts of deformed sedimentary rocks. The distortion of earlier structures in these lineaments suggests that their formation was associated with transcurrent displacements of up to a few hundred km[5].

Plotting of the north-easterly lineaments on a globe shows that they fall on small circles about an axis lying near the west end of the Aleutian arc. Regional dyke-swarms dated at (2,500) 1,800 Myr in the Slave province and the Ungava peninsula of the Superior province[8,9] lie close to a great circle passing through a point only some 20° from this axis and may record extensional rotation. If we regard these displacements as adjustments to the bodily motion of the continental sheet, it seems reasonable to infer that the sheet was being rotated about the same axis. A clockwise movement parallel to the small circles defined by the transcurrent lineaments seems compatible with the appropriate polar wander path (track 5, 2,500–1,950 Myr) of Donaldson *et al.*[2] Figure 1 shows that movements parallel to the lineaments would be capable of opening or closing oceanic basins on the sites of the Labrador and Coronation geosynclines which are set at an angle to them. We therefore envisage a situation in which the precursor of North America moved westward, perhaps in response to the opening of a major ocean on its north-eastern flank and was simultaneously distorted by displacements on deep transcurrent faults and by the jostling of the stable provinces against their more mobile surroundings. The short eugeosynclinal welts may mark 'tears' linked by north-easterly transform faults, along which small oceans were formed and subsequently destroyed. A certain amount of further internal deformation was taken up in those portions of the Churchill province which are occupied neither by geosynclinal assemblages nor by lineament tracts and which were sites of metamorphism and granite formation during the Hudsonian event at about 1,800 Myr. The survival of incompletely modified older tectonic patterns in some of these regions, as indicated by the aeromagnetic map[10], suggests however that deformation was relatively weak.

Distortion and metamorphism continued in the interior of the North American continental sheet until the ending of the Hudsonian event, perhaps changing in style or direction at the time at which the hairpin bend of the polar wander curve[2] suggests a change in motion of the sheet as a whole. Structures of new kinds developed from about 1,300 Myr onward include the rift-like Keweenawan and related volcanic troughs, the Muskox basic complex, the Mackenzie dyke-swarm and the Belt sedimentary basin. Taken together with the Gardar igneous province of southern Greenland, these features—mostly suggesting extensional movements—define an incomplete polygonal pattern avoiding the oldest stable massifs. The nearest analogies seem to be with Phanerozoic rift-valley systems; the polygonal pattern suggests fracturing over domal upwarps around the Great Lakes and Coronation Gulf. Mobile provinces were less extensive than in the preceding period and there is some evidence[2] suggesting that the principal (Grenville) mobile belt was peripheral rather than internal in position.

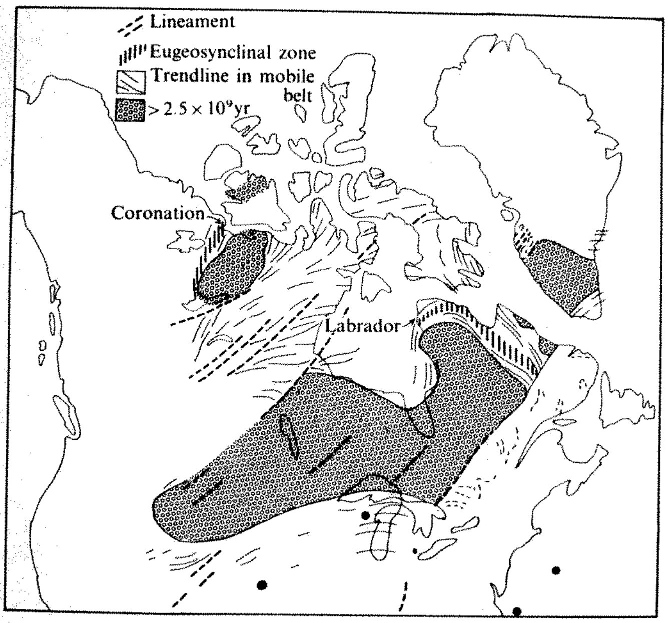

FIG. 1 Some Proterozoic structures in North America and Greenland.

Greenland and north-west Britain

The Pre-Ketilidian stable massif of Greenland is enclosed by mobile regions including the Ketilidian and Nagssugtoqidian of Greenland and the Laxfordian of Britain. Bridgwater *et al.*[11] and Windley[12] infer that the massif moved relative to its mobile borders and recognise contrasts in its marginal relationships similar in implications though not in detail to those discussed above. On a construction which eliminates the North Atlantic and Davis Strait (Fig. 1) the great shear zones at the north-west side of the Pre-Ketilidian massif fall into place as members of the set of north-easterly transcurrent lineaments developed in North America. The north-westerly shear zones of the Laxfordian complex clearly do not belong to this set which may be represented in Scotland by a steep north-easterly zone of strong deformation which skirts the Outer Hebrides. No eugeosynclinal welts oblique to the principal lineaments are represented. Contrasts between the Ketilidian mobile belt of southern Greenland and the Laxfordian and Nagssugtoqidian provinces suggest that the former, which has been compared with the Andean belt[11], may be part of a peripheral system and the latter parts of an internal ensialic network.

Gondwanaland

So much of the early Proterozoic provinces in Gondwanaland has been reworked during Mozambiquian or Indian Ocean tectonic events (at about 800-500 Myr) that their early history is obscure. Some structures shown in Fig. 2 are usually regarded as not older than late Proterozoic and may ultimately have to be discarded for the purposes of this discussion. We provisionally recognise in Africa a set of major Proterozoic lineaments including the Ubendian belt, long regarded by McConnell as a product of transcurrent movement[13], the Aswa zone of Uganda and perhaps the great dislocations of the Hoggar. These lineaments fall on small circles about an axis located near the north-east of peninsular

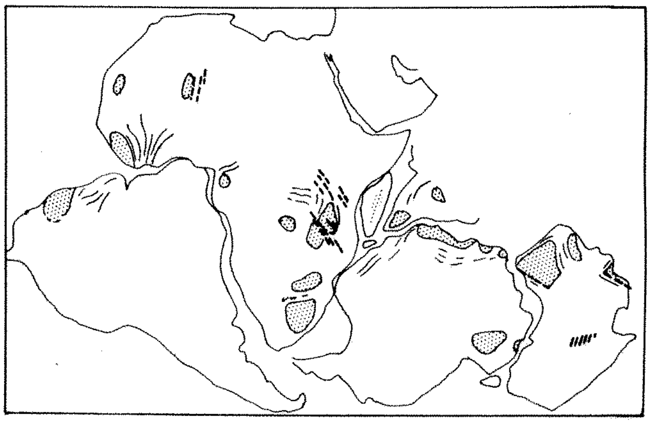

FIG. 2 Some Proterozoic structures in Gondwanaland; symbols as in Fig. 1.

India. Clockwise motion of the continent on this axis would be roughly consistent with the polar wander track for the period 2,340-2,200 Myr[1].

In Australia, narrow north-easterly structures comparable with the transcurrent lineaments of other continents make the boundaries of the Yilgarn and Carpentaria stable blocks along the Frazer Range and the Halls Creek mobile belt. On Smith, Briden and Drewry's reconstruction of Gondwanaland[14] they fit reasonably well on the small circles defined by the African lineaments, as does the main early Proterozoic metamorphic belt of Antarctica[15]. These coincidences are consistent with the inference drawn from palaeomagnetic studies[3,16] that the core of Gondwanaland moved as a unit through much of Precambrian time. Mobile belts such as that of Mount Isa which have geosynclinal characters are, as in North America, oblique to the lineament set and may mark the site of oceans opened by small relative movements of adjacent blocks.

The structural relationships discussed above seem to record an integrated series of relative movements taking place within one or a few large continental sheets which were themselves moving as essentially coherent units. Peripheral mobile belts formed in response to bodily movements of the sheets may be distinguished from interior, ensialic, mobile belts formed in response to comparatively small relative movements of the more stable massifs within them. Deformation in the interior mobile belts together with displacements on major sets of transcurrent lineaments which traversed the continents and acted in some instances as transform faults led to distortion of each continental sheet as a whole and to the opening and closing of small oceanic basins within it. Metamorphism and granite formation widely associated with tectonic activity show that geothermal gradients in the interior network of mobile belts were steep.

The distinctive features of the early Proterozoic tectonic systems are those which record the internal deformation of the large continental plates. Taken in conjunction with the evidence of bodily movements supplied by palaeomagnetic data they allow, as we suggest above, interpretation of Proterozoic tectonics on a global scale.

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Methionine transfer RNAs associated with avian oncornavirus 70S RNA

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tRNA free in RNA tumour viruses contains very little tRNA$_f^{Met}$, whereas tRNA associated with viral 70S RNA contains both tRNA$_m^{Met}$ and tRNA$_f^{Met}$. The close association of the initiator tRNA with 70S nucleic acid suggests that it may be involved as a primer for reverse transcriptase.

RNA tumour viruses contain two physically separate classes of tRNA. The major class is free within the virion, and contains a selected population of host cell molecules[1–6]. The minor class of 4S RNA is intimately associated with viral 70S RNA, and comprises 2.5–3.0% of the total mass of the aggregate[7]. This 70S-associated tRNA has a minor base composition similar to that of free viral 4S RNA[8], and contains several amino acid acceptor activities including acceptance of lysine, phenylalanine, proline, histidine, threonine and serine[9].

Eukaryotic cells contain two types of tRNA which accept methionine. One species is the initiator of protein synthesis and although it is not formylated *in vivo* (as in the initiator tRNA in prokaryotes) it can be formylated by *Escherichia coli* transformylase. It is therefore designated tRNA$_f^{Met}$. The other tRNA^{Met} species donates its amino acid into internal positions of proteins and is designated tRNA$_m^{Met}$[10].

We have previously analysed the methionine-acceptor activity of the free tRNA of avian myelobastosis virus (AMV), and shown that 85% of this fraction consists of a single 'm' type species, similar to a corresponding chick cell molecule with respect to chromatography on RPC-5 absorbent, the electrophoretic mobility of the terminal methionyl-oligonucleotide, and its activity in donating methionine into polypeptides synthesised in an ascites cell-free system[11]. Free viral tRNA contains very little initiator RNA.

We now report a similar study of 70S-associated tRNA^{Met}, and show that this fraction contains both types of methionine-accepting tRNA. Reversed-phase chromatography on RPC-5 of 70S-associated tRNA charged with ^{35}S-methionine reveals a profile qualitatively similar to that obtained with chick cell tRNA$^{\mathrm{Met}}$, containing one peak of 'f' type activity, peak I, and three peaks of 'm' type tRNA$^{\mathrm{Met}}$, peaks II, III and IV[11]. Peak I isolated from AMV 70S-associated tRNA is shown to be an initiator; it can be formylated by *E. coli* transformylase the terminal methionyl-oligonucleotide co-electrophoreses with that from Met-tRNA$_{\mathrm{f}}$ of chick and ascites cells, and it responds to the synthetic polymer AUG (U)$_{35}$ in an ascites cell-free system. Furthermore, 70S-associated Met-tRNA$_{\mathrm{f}}$ donates its methionyl residue into the N-terminal position of nascent peptides synthesised *in vitro* in response to both natural and synthetic mRNAs. The 70S-associated Met-tRNA from peak IV, on the other hand, is identical to the free virion peak IV material and has been characterised as a tRNA$_{\mathrm{m}}^{\mathrm{Met}}$ species.

Structure of 70S-associated Met-tRNAs

AMV was purified from the serum of leukaemic 10 to 15-d-old chicks, and Rous sarcoma virus, Prague strain (RSV (Pra)) and Schmidt-Rupin strain (RSV (SR)) were prepared from the supernatant medium of infected secondary chick embryo cells. 70S RNA was purified by published procedures[1,7]. 4S RNA was released from the 70S RNA by heating to 70° C for 3 min in 10 mM EDTA, 100 mM Tris HCl, *p*H 7.2. The solution was chilled, and the 4S RNA separated by sucrose gradient centrifugation. Aminoacyl-tRNA synthetases were prepared from chick embryos by the method of Taylor *et al.*[2] and freed of endogenous tRNA by passage through Sephadex G100. 70S associated RNA was acylated *in vitro* with ^{35}S-methionine (Radiochemical Centre, specific activity 100 Ci mmol^{-1}) and extracted as previously published. Deacylation of the viral tRNA prior to reaction with amino acyl-tRNA synthetase was found to be unnecessary. The separation and isolation of ^{35}S-Met-tRNA using RPC 5 absorbent (Miles Laboratories) have been described[11].

RPC-5 chromatographic profiles of AMV, RSV (Pra), and RSV (SR), 70S-associated RNA charged with methionine are qualitatively similar to the profiles shown by free viral Met-tRNA, chick embryo and myelobast Met-tRNA (Fig. 1). In each case there are four peaks present and co-chromatography using ^{3}H and ^{35}S double labels shows that the corresponding species are chromatographically identical (data not shown). Quantitatively, there are distinct differences. Viral free tRNA that is not associated with 70S RNA shows a predominance of only one species, peak IV, which resembles chick embryo peak IV with respect to both structure and function. Peak IV is an m type of tRNA$^{\mathrm{Met}}$, and donates its methionyl residue into internal positions of polypeptides synthesised *in vitro*[11]. In contrast, viral Met-tRNA that is part of the 70S aggregate contains, in addition to peak IV, significant amounts of peak I, which in chick and mouse cells functions as the initiator tRNA[11]. 70S-associated Met-tRNA also contains a lesser amount of peak III. A similar chromatographic profile of 70S-associated Met-tRNA was obtained with each of the viruses tested (AMV, RSV(Pra) and RSV(SR)).

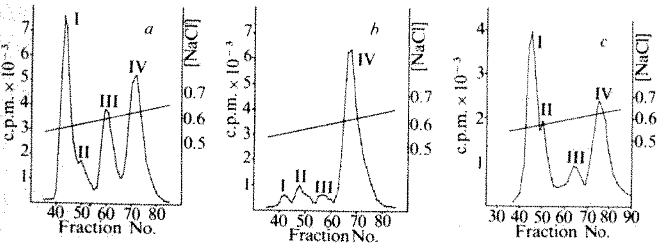

Fig. 1 RPC-5 chromatography of ^{35}S-Met-tRNAs from, *a*, chick embryo; *b*, AMV-free 4S RNA; *c*, AMV 70S-associated 4S RNA. 10^{5} c.p.m. of each aminoacyl-tRNA were applied to a 0.9 × 30 cm column of RPC-5. The isoaccepting species were eluted with a linear gradient of 0.5–0.7 M NaCl (total volume 100 ml) in 10 mM NaAc, 10 mM MgCl$_2$, 2 mM 2-mercaptoethanol at *p*H 4.5. The flow rate was 60 ml h^{-1} and 0.75 ml fractions were collected and counted in a scintillation fluid containing PPO, POPOP, and Triton X100 (refs 11, 20).

Table 1 Incorporation of ^{35}S-methionine from AMV Met-tRNA species in response to AUG(U)$_{35}$

	^{35}S-methionine incorporated (c.p.m.) No additions	20 μg AUG(U)$_{35}$
70S-Associated peak I	834	6,661
70S-Associated peak IV	1,480	967
Free peak IV	1,211	1,459

Protein synthesis reactions were performed in a cell free system derived from mouse ascites cells[11,14] in a total volume of 25 μl containing 40,000 c.p.m. of the appropriate ^{35}S-Met-tRNA. The Mg^{2+} concentration was 5 mM, the K^{+} concentration 80 mM; incubation was for 20 min at 37° C.

The two classes of eukaryotic Met-tRNA have different nucleotide sequences near the amino acid acceptor end and digestion with ribonuclease T$_1$ liberates methionyl oligonucleotides that can be separated on electrophoresis at *p*H 3.5[10,11]. In experiments not shown here, the terminal oligonucleotides of 70S-associated Met-tRNA I–IV were examined. Peak I oligonucleotide co-migrates with the corresponding oligonucleotide from ascites and chick cell Met-tRNA$_{\mathrm{f}}$. Peak II contains both f and m terminal oligonucleotides, and peaks III and IV have a 3′ OH terminus identical to ascites and chick cell Met-tRNA$_{\mathrm{m}}$.

As mentioned above, one of the distinguishing features of the initiator tRNA is its ability to accept a formyl group. Although transformylase activity is not present in the cytoplasm of eukaryotes, Met-tRNA$_{\mathrm{f}}$ from these cells can react with *E. coli* transformylase to give fMet-tRNA$_{\mathrm{f}}$[10,12]. This can then be identified by the electrophoretic mobility of the terminal formyl-methionyl-adenosine, which is liberated on digestion with pancreatic ribonuclease. Figure 2 shows that when 70S-associated Met-tRNAs I–IV are treated in this manner only peak I (and peak I material which is a contaminant of peak II) is formylated. This species is therefore related to eukaryotic and bacterial initiator tRNA.

Function of 70S-associated Met-RNAs

The coding properties of tRNA$_{\mathrm{f}}^{\mathrm{Met}}$ and tRNA$_{\mathrm{m}}^{\mathrm{Met}}$ differ in that tRNA$_{\mathrm{m}}^{\mathrm{Met}}$ recognises only internal AUG codons, whereas the initiator responds to either AUG or GUG at or near the 5′ end of synthetic messengers[10,13]. This difference can be detected by measuring the incorporation of methionine from the different species in response to synthetic mRNAs. The activity of viral 70S-associated Met-tRNAs in response to AUG(U)$_{35}$ was tested in a mouse ascites cell-free system. The results in Table 1 show that peak I responds markedly to this polymer and demonstrate its ability to function as an initiator tRNA *in vitro*. Peaks IV from both 70S-associated and free viral Met-tRNA are much less active, as is usual for tRNA$_{\mathrm{m}}^{\mathrm{Met}}$ species[10,13].

In eukaryotes, all nascent polypeptides are thought to contain an N-terminal methionyl residue, donated by the initiator tRNA. In most cases, this methionine is subsequently cleaved from the growing peptide chain, presumably by a methionine amino-peptidase[10,14]. Cleavage can be inhibited by preventing the nascent peptide from becoming sufficiently long to be a substrate for the cleavage enzyme. This can be achieved by use of the antibiotic sparsomycin which inhibits chain elongation but has little effect on chain initiation[15]. Thus, in the presence of the antibiotic short chain initiation peptides accumulate which are labelled with methionine exclusively from Met-tRNA$_{\mathrm{f}}$. Figure 3 shows an autoradiograph of peptides made in the presence of sparsomycin and separated on electrophoresis at *p*H 3.5. 70S-associated peak I donates methionine into the

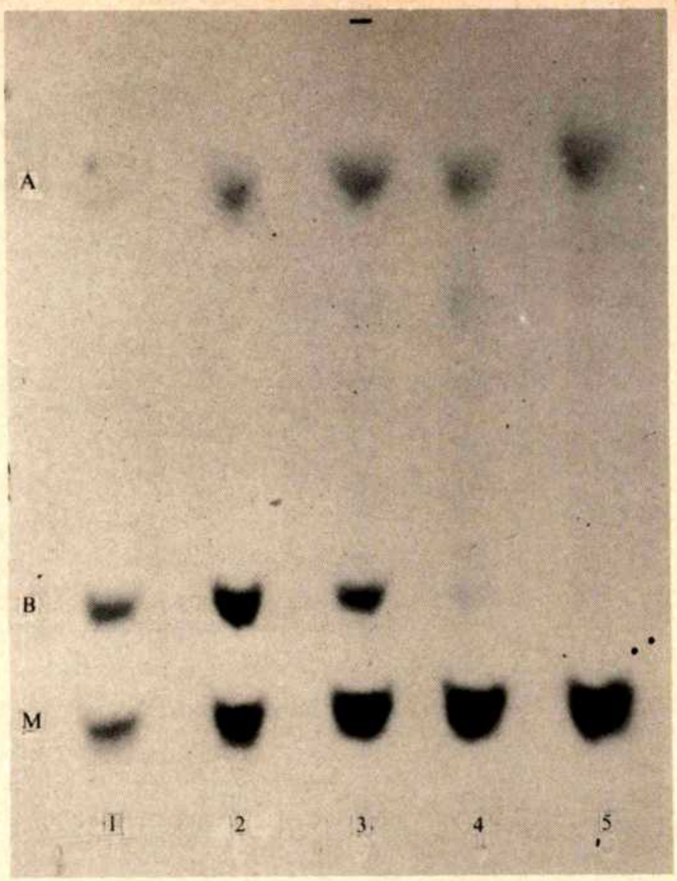

Fig. 2 Pancreatic ribonuclease digest of separated ^{35}S-Met-tRNAs after reaction with *E. coli* transformylase and formyl donor. Formylation reactions (25 μl) were carried out in an incubation mixture containing 100 mM Tris HCl, *p*H 7.5, 10 mM $MgCl_2$, 10 mM KCl, 6 mM ATP, 3 mM CTP, 6 mM 2-mercaptoethanol, 0.5 mg ml^{-1} *E. coli* supernatant fraction (which had been concentrated by ammonium sulphate precipitation), 0.25 mM calcium leucovorin and 10,000 c.p.m. of purified peaks I–IV isolated from AMV 70S RNA. Incubation was continued for 5 min at 37° C, one drop of pancreatic ribonuclease A (200 μg ml^{-1} in H_2O) added, and the mixture immediately applied to Whatman 3 mm paper for electrophoresis at *p*H 3.5 (60 V cm^{-1}, 30 min). The dried paper was autoradiographed for 2 weeks with Osray M film. 1, Chick peak I; 2, AMV 70S associated peak I; 3, peak II; 4, peak III; 5, peak IV. A, methionyl adenosine; B, formyl-methionyl adenosine; M, free methionine. Note that during incubation at 37° C, those species of Met-tRNA that are not enzymatically formylated are to some extent chemically deacylated and give free methionine rather than methionyl adenosine.

specific initiation peptides made in response to both encephalomyocarditis virus RNA (EMC RNA) and $\text{AUG(U)}_{\overline{35}}$. Free viral peak IV, on the other hand, is inactive in labelling sparsomycin peptides.

The experiments described above show that 70S-associated Met-tRNA peak I is related to normal chick initiator tRNA in both structure and ability to take part in protein synthesis. In experiments similar to those already reported for free viral Met-tRNA peak IV, we have confirmed that 70S-associated peak IV is active in donating methionine into polypeptide synthesised in response to EMC RNA (Table 2).

Other experiments which will be published in detail elsewhere have shown that the polypeptide synthesised in response to EMC RNA and labelled with methionine from different Met-$tRNA_m$ species, is identical no matter whether the tRNA used was from mouse or chick, or free AMV or 70S RNA-associated fractions. Furthermore we can detect no difference in the peptide labelling pattern obtained using Met-tRNA from either peak III or peak IV.

Wang, Kothari, Taylor and Hung (personal communication) have also shown that the free AMV Met-tRNA of peak IV is not an initiator species.

Table 2 Incorporation of ^{35}S-methionine from chick and AMV Met-tRNA species in response to EMC RNA

	^{35}S-methionine incorporated (c.p.m.) No additions	Plus 2 μg EMC RNA
Chick peak I	522	1,233
Chick peak III	2,042	9,236
Chick peak IV	2,136	11,028
AMV free peak IV	2,059	10,210
AMV 70S associated IV	1,567	7,921

Incubation conditions were exactly as described in Table 1, except that the Mg^{2+} concentration was 3 mM and the K^+ concentration 120 mM.

Role of viral tRNAs

$tRNA_f^{Met}$ is required for the initial step in protein synthesis. There are many properties of the eukaryotic initiator tRNA that distinguish it from normal tRNAs, including the reaction with bacterial methionyl-tRNA synthetase[16], and transformylase[12], the recognition of initiation factors[17], the inability to form a stable complex with elongation factors[18], and the unusual

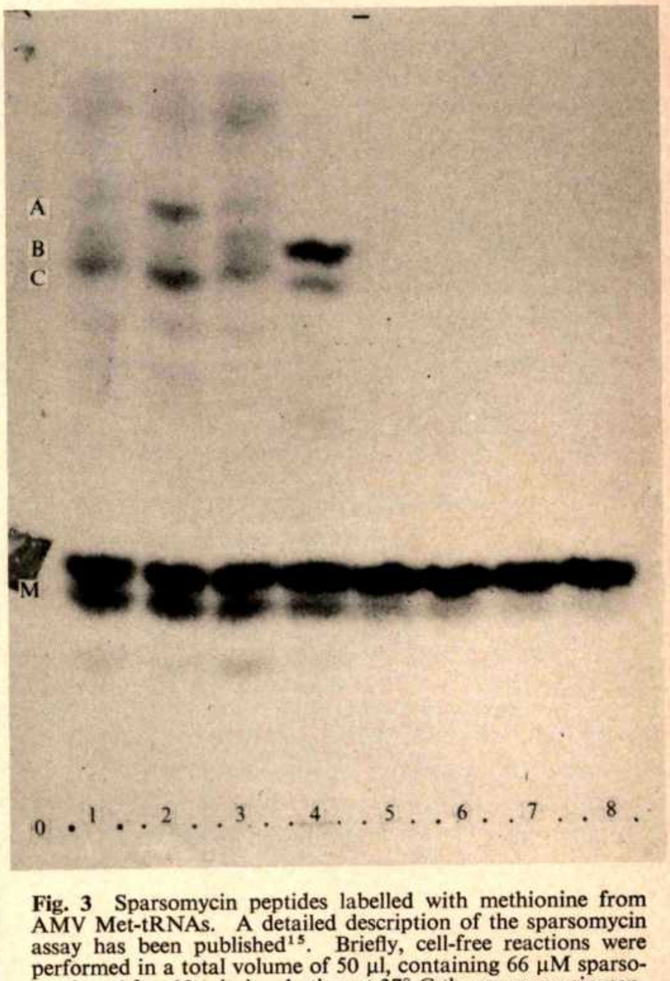

Fig. 3 Sparsomycin peptides labelled with methionine from AMV Met-tRNAs. A detailed description of the sparsomycin assay has been published[15]. Briefly, cell-free reactions were performed in a total volume of 50 μl, containing 66 μM sparsomycin. After 10 min incubation at 37° C the sparsomycin peptides were isolated, separated by electrophoresis at *p*H 3.5 (60 V cm^{-1}, 75 min) and visualised by autoradiography (10 d, Osray M film). Incubations 2 and 6 included 3 μg EMC RNA, 4 and 8 40 μg $\text{AUG(U)}_{\overline{35}}$, 1–4 contained 50,000 c.p.m. AMV 70S-associated Peak I, Met tRNA; 5–8 55,000 c.p.m. AMV free peak IV Met-tRNA. 1, 2, 5 and 6 were at 3 mM Mg^{2+} and 120 mM K^+; 3, 4, 7 and 8 at 5 mM Mg^{2+} and 80 mM K^+; all other ingredients were as published[11,15]. A, Met Ala; B, Met Phe; C, Met Ala Thr; M, free methionine and methionine sulphoxide; O, origin.

coding properties[13]. All these properties strongly suggest that the unique structure of tRNA$_f^{Met}$ has been specially adapted for its role in the initiation of protein synthesis. The presence of this unusual molecule in close association with tumour virus nucleic acid is therefore somewhat surprising. 4S RNA molecules are required as primers for reverse transcriptase which synthesises viral DNA[19]. Although viral tRNA$_f^{Met}$ seems to be identical to the host cell molecule both in its structure and its ability to function as an initiator tRNA, it is possible that tRNA$_f^{Met}$ is also involved as a primer in the transcription process. It is equally possible that another of the 70S-associated 4S RNA molecules, including the other methionine accepting species, might be involved as a primer. The role of those tRNA species that are not primer molecules is unclear, but they may be necessary to maintain the structure of the genome, or be an example of tRNA modulation[21]. A definite answer to these questions must await further analysis of the structure of the primer molecule and the role of virus-associated tRNAs in the translation of tumour virus messenger RNA.

Contaminating degradation products of the high molecular weight RNA may also be released by denaturation of 70S RNA. Hence, it is difficult to calculate the number of molecules of tRNA^{Met} associated with the 70S RNA. A crude estimate indicates that there may be about one mol of methionine acceptor activity per mol of 70S RNA. Since the amount of free 4S RNA in different virus preparations is variable, a calculation of the amount of free tRNA^{Met} has little meaning. In general, however, there is usually five to ten times more tRNA^{Met} free in the virion than associated with 70S RNA. The significance of the distribution of tRNAs between viral compartments is, as yet, unexplained.

The origin of the viral tRNAs is also uncertain but the similarity in structure and function between host and both free and associated viral methionine accepting tRNAs strongly suggest that the viral tRNAs are of host cell origin and are not virus coded.

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Lateral diffusion of rhodopsin in the photoreceptor membrane

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Rhodopsin undergoes rapid lateral diffusion in the disk membranes of isolated frog and mudpuppy rods. The rate of lateral diffusion is consistent with the rapid rotational diffusion of rhodopsin: both indicate the disk membrane is highly fluid with a viscosity of ~1P.

HIGH-speed flash photometry has shown that rhodopsin undergoes rapid rotational diffusion in the disk membrane of frog rod outer segments[1]. Rotational diffusion about an axis perpendicular to the membrane occurs with a relaxation time about 20 μs at 20°C. Rhodopsin is deeply embedded in the membrane, and this relaxation time indicates that the site it occupies is highly fluid, with an effective viscosity for rotational diffusion comparable with that of a light oil. Rhodopsin molecules appear from X-ray evidence to be distributed in a liquid-like array in the plane of the membrane[2], so they may also be free to undergo rapid lateral diffusion in the membrane. Recently, evidence has been reported for several types of cells that membrane proteins can undergo rapid lateral motion[3-6,6a], and it is clear that a general understanding of molecular events within cell membranes will require more knowledge of the diffusional motions of membrane proteins. We report here microspectrophotometric observations of rapid lateral diffusion of rhodopsin in freshly isolated rod outer segments.

Rod outer segments were obtained by gently shaking retinas dissected under dim red light from the eyes of frog (*Rana catesbeiana*) and mudpuppy (*Necturus maculosus*) which had been dark-adapted for more than 10 h. The rods were shaken into a microchamber containing a standard Ringer solution and examined in a Shimadzu 50L microspectrophotometer (MSP) fitted with a high quantum efficiency photomultiplier (Hamamatsu type R375). Single rods which appeared intact and which lay flat on the bottom of the chamber were selected for observation, and all observa-

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tions were completed within 30 min after the rods were isolated. The rhodopsin in isolated rods, once bleached, does not regenerate, hence dim red light was used for selection. focusing, and alignment.

The measuring beam of the MSP was limited by an aperture and a condensing lens to form a rectangle about 2 × 20 μm in cross section. The long axis of the rectangle was aligned with the long axis of the rod and a simple motor-driven 'alternator' optically shifted the rectangular measuring beam back and forth between the two sides of the rod. Thus the absorbance of the rhodopsin on each side of the rod could be compared directly. The wavelength of the measuring beam was set at the absorption peak of the visual pigment: 500 nm for frog, 530 nm for mudpuppy.

With suitable alignment and focusing the absorbance was essentially equal on both sides of unbleached rods, as shown by the first pair of measurements on an unbleached rod at the beginning of each of the two recordings in Fig. 1. The alternator was then stopped momentarily and the intensity of the measuring beam was increased about 1,000-fold to bleach some pigment on one side of the rod. The exponential decrease in absorbance during the bleach was recorded, and then the intensity was dropped to the original level and the alternator turned on again. Figure 1 shows that immediately after the bleach the absorbance on the unbleached side was little changed, but there was a marked drop in absorbance on the bleached side. Within the next few seconds, however, the absorbance of the unbleached side decreased while that on the bleached side increased, and within less than 1 min the absorbance of the two sides became equal, reaching a final level midway between that of each side immediately after the bleach. This is expected if rhodopsin molecules can diffuse laterally in the membrane: the total pigment present after the bleach remained constant and only its distribution changed, approaching uniform distribution with an exponential time course. The half-time of the final approach to uniform distribution has been observed in more than ten rods each for frog, and mudpuppy. At 20°C, it was 35 ± 7 s for frog rods 8 μm in diameter, and for mudpuppy rods 23 ± 5 s for rods 12 μm in diameter. In addition, half-times measured at temperatures in the range 17°–24°C decreased with temperature with a Q_{10} of 3.7 ± 0.7 in frog rods and 3.1 ± 1.4 in mudpuppy rods (apparent energy of activation 24 and 21 kcalories mol^{-1} respectively).

Control experiments were performed to help ensure that the observed redistribution of rhodopsin was the result of diffusion of the entire rhodopsin molecule and not simply energy transfer between chromophores or chromophore transfer between protein molecules (opsin). As Fig. 2 shows, there was no redistribution of rhodopsin in rods fixed with glutaraldehyde before bleaching. In contrast, fixation with the same amount of formaldehyde had no effect on the rate of redistribution. These results are consistent with the findings that glutaraldehyde fixation blocks rotational diffusion of rhodopsin whereas formaldehyde fixation has no effect[1,7]. Glutaraldehyde seems to form crosslinks between rhodopsin molecules whereas formaldehyde does not, for rhodopsin can be extracted with digitonin after formaldehyde fixation but cannot be extracted after glutaraldehyde fixation[7]. Chromophore transfer between opsin molecules seems unlikely given the nature of the covalent linkage between retinal and opsin, but as a further check experiments were performed in the presence of 50 mM hydroxylamine which rapidly converts free chromophore to the stable compound retinal oxime (λ_{max} = 363 nm)[8]. When one side of the rod was bleached, all chromophores from the bleached pigment converted immediately to retinal oxime. The redistribution of rhodopsin, however, proceeded as usual, no further oxime was formed and the total remaining pigment stayed constant. Thus the chromophores of the unbleached pigment did not react with hydroxylamine, as might be expected if transfer of free chromphores accounted for the redistribution of pigment. Energy transfer between rhodopsin molecules is also improbable since the distance between chromophores is large[9,10], and the fluorescence yield is low[11]. Moreover, energy transfer cannot explain the redistribution of pigment since pre-lumi rhodopsin, the first photoproduct after bleaching, forms within a few picoseconds at room temperatures[12]. Thus energy transfer between rhodopsin molecules, if it occurs, would have to take place on a sub-picosecond scale, whereas the observed redistribution takes place in seconds. Finally, as a further test for the plausibility of energy or chromophore transfer, we investigated whether redistribution of pigment occurs along the axis of the rod. Rods were bleached with a black and white 'grating' pattern which alternated in intensity along the axis of the rod with a repeat distance of 8 μm. Rhodopsin absorbance was monitored by scanning the length of the rod with the measuring beam in this case being limited to a circular spot 4 μm in diameter. After the bleach the resulting 'grating' pattern in absorbance persisted unchanged for as long as stable recordings could be made (~10 min)[13]. Thus the redistribution of rhodopsin seems to be confined to the plane of the disk membrane, since no migration along the length of the rod was detected. This result is consistent with the autoradiographic investigations reported by Young and his co-workers[14] which show that once rhodopsin is inserted into the membrane of a disk, it does not migrate away from that disk. From all these considerations, lateral diffusion of entire rhodopsin molecules within the disk membrane seems to be the only reasonable explanation for the redistribution of pigment shown in Fig. 1.

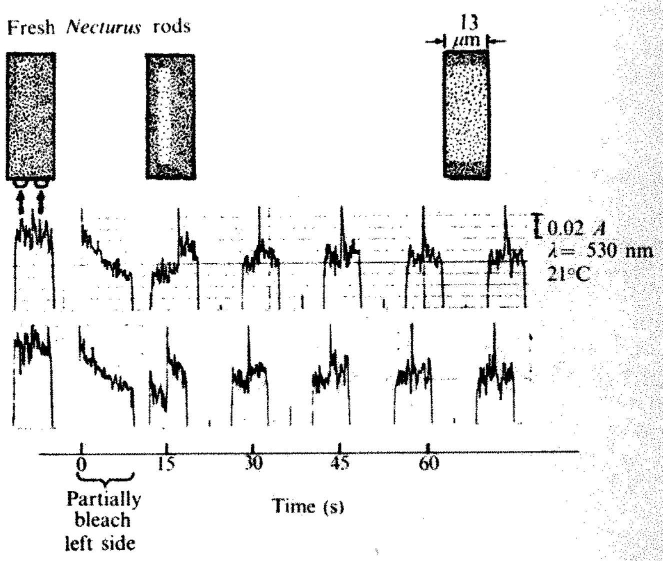

FIG. 1 Lateral diffusion of rhodopsin in the disk membrane. The diagrams of a rod depict the pigment distribution corresponding in time with the absorbance measurements shown below. The arrows indicate the location on the rod at which each absorbance measuremennt was made. Recordings made from two different rods are shown to give an indication of the repeatability of the measurements. In each experimennt the chart recorder was run continuously, as shown by the time base. The alternator also ran continuously except during the bleach. The records thus consist of a repeated pattern in which absorbance measurements were made first on the left side, then the right side of the rod. Between each pair of measurements baseline measurements were also made to ensure that no drifts occurred (for clarity, these were omitted from the figure). The spikes on the traces were caused by switching transients in the alternator. The diameter of a disk is essentially equal to the width of the rod, and the width was measured in the MSP after completing each experiment. In a separate experiment described in the text[13], it has been shown that no diffusion of rhodopsin occurs along the length of the rod. Thus in the diagrams of a rod, the rhodopsin concentration is depicted as unchanged at the ends, where no bleaching occurred.

The half-time for redistribution of rhodopsin did not seem to depend on the amount of uniform bleaching which in some cases occurred before starting the diffusion experiments. Redistribution proceeded as usual even when two-thirds of the pigment was previously bleached. Since rhodopsin molecules occupy a major fraction (~⅓ to ⅔) of the total membrane area, redistribution can only occur if rhodopsin molecules displace bleached molecules (opsins). Thus opsin molecules appear to have essentially the same diffusion constant.

The diffusion constant, D, for lateral diffusion of rhodopsin in the disk membrane can be obtained by solving the two-dimensional diffusion equation for the closed boundary defined by the edge of the disk. The result is, to first order of accuracy,

$$D = (0.69bL^2)/(\pi^2 t_{1/2}) \quad (1)$$

where L is the diameter of the disk $t_{1/2}$ is the half-time with which pigment distribution approaches final uniform distribution, and b is a geometry factor included to account for the irregular boundary of the disk, especially the fissures which extend well in toward the centre. To obtain the value for b, we performed an analogue experiment in which the rate of diffusion of heat was observed in thin sheet-metal disks with the boundary cut to simulate the shape of typical disks in frog and mudpuppy rods as observed by electron microscopy[15]. The value obtained was 2.7 ± 1.0 for frog disks which have numerous deep fissures, and 0.9 ± 0.2 for mudpuppy disks which have numerous but very shallow fissures. For these values of b, and the observed values of L and $t_{1/2}$, equation (1) yields

$$D = (3.5 \pm 1.5) \times 10^{-9}\ \mathrm{cm^2\,s^{-1}} \quad \text{for frogs}$$

$$D = (3.9 \pm 1.2) \times 10^{-9}\ \mathrm{cm^2\,s^{-1}} \quad \text{for mudpuppy}$$

Viscosity of membrane

The diffusion constant is a measure of diffusion rate, depending not only on the viscosity of the medium in which diffusion occurs, but also on the size of the diffusing particle. Therefore, even within a given medium diffusion constants for various particles cannot be compared directly. However, as Einstein showed, the viscosity of a solution can be predicted by observing the diffusion constant of a large solute molecule in the solution and by assuming that in undergoing thermal motions the solute molecule experiences a viscous drag given by Stoke's law[16]. Later theoretical treatments have shown that only a minor numerical correction is needed to extend this procedure to molecules with dimensions comparable with those of the solvent molecules[17]. Thus, although viscosity is usually observed macroscopically, it is a property of solutions which can be observed equally well using small molecules, even molecules as small as the solvent molecules. In membranes, diffusion constants have been observed for various probes using different techniques. It seems reasonable to expect that regardless of the size and shape of a probe molecule or structure, as long as a significant portion of it spans a major fraction of the membrane thickness, its rate of diffusion in the plane of the membrane, when properly interpreted, should yield the same viscosity. Hence diffusion constants for different probes can be compared by comparing the predicted viscosities.

The rotational diffusion of rhodopsin, as previously reported[1], indicates the viscosity, η, of the membrane site occupied by frog rhodopsin is about 2 P at 20°C. The calculation was based on the relationship derived by Einstein for Brownian rotation of a spherical particle about one axis in a isotropic medium. A similar calculation can be made for lateral diffusion by using the Einstein-Sutherland diffusion equation[17]

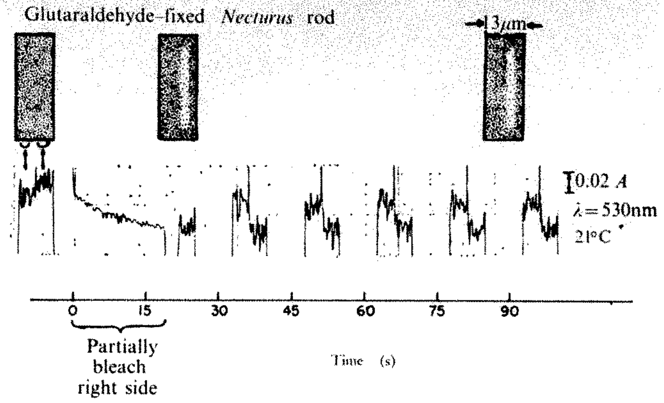

Fig. 2 Glutaraldehyde fixation blocks lateral diffusion of rhodopsin in rod disks. Isolated mudpuppy rod outer segments were soaked in 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.1) for 15 min before starting the experiment. The diagrams again depict the distribution of pigment at the time of measurements shown below. Although the recording procedures were the same as described in Fig. 1, after glutaraldehyde fixation the unequal distribution produced by the bleaching persisted as long as stable recordings could be made. It may be noted in this experiment that stray light caused negligible bleaching on the unbleached side. Hence in the first recordings after the bleach in Fig. 1, the small decrease in absorbance on the unbleached side is the result of some diffusion having already occurred.

$$\eta = (kT)/(a\pi Dr) \quad (2)$$

where k is the Boltzman constant, T is the absolute temperature, r is the radius of a spherical diffusant, and $a = 6$ for diffusants large compared with the solvent molecules, while $a = 4$ for diffusants with dimensions comparable with the solvent molecules. Recent evidence indicates that the rhodopsin molecule spans the membrane[18,19] (see also discussion of ref. 13) and on the basis of molecular weight[20] and X-ray evidence[9], the effective radius of rhodopsin for lateral diffusion is probably in the range 15–30 Å. This is significantly larger than the effective radius of phospholipids for lateral diffusion. In this case equation (2) yields a viscosity of about 1–4 P. This is in the same range indicated by the rotational diffusion of rhodopsin, 0.7–6 P[1]. Thus if it is assumed that rhodopsin has an effectively circular cross section, the viscosity of the disk membrane indicated by lateral diffusion is in good agreement with the viscosity indicated by rotational diffusion. This is not surprising since lateral diffusion in the membrane and rotational diffusion about an axis perpendicular to the membrane surface both depend on the same 'slippage' motion between the surface of the rhodopsin molecule and the molecules in the surrounding membrane environment. Moreover, as we show when, each rhodopsin molecule undergoes numerous collisions with other rhodopsin molecules during its rotational relaxation time. Thus a rhodopsin molecule samples essentially the same membrane environment for both its rotational and translational motions.

Evidence that other membrane proteins may also exist in such highly fluid environments has been reported by Frye and Edidin who observed, using fluorescent antibodies, the translational diffusion of cell surface antigens in membranes of mouse and human cell hybrids[3]. The rate at which the surface antigens diffuse along the cell surface indicates the membrane viscosity for lateral diffusion of these proteins is on the order of 1–10 P at 20°C. (For a recent discussion of membrane fluidity, see ref. 21.) As pointed out previously[1], lipid soluble spin labels seems to encounter a viscosity of 0.1–1 P at 20°C in the lipid phase of mitochondria and nerve axons[22,23]. Spin label studies by Scandella *et al.* on lateral diffusion of phospholipids in sarcoplasmic reticulum vesicles from rabbit muscle[24] have shown the diffusion constant to be about 6×10^{-8} cm² s⁻¹ at 40°C. Using the Einstein-Sutherland equation for self-diffusion, a reasonable extrapolation of this result to 20°C indicates that membrane viscosity falls within 0.5–5 P. Thus inspite of differences in probes and techniques the viscosity of each of these membranes seem to lie within an order of magnitude of 1 P. That is, the viscosity for lateral motion in each membrane is between 10 and 1,000 times the viscosity of water. Differences in the composition of each membrane and in the membrane layer sampled by the probe molecule, together with possible systematic experimental errors, can account for the variations in viscosity so far observed. Rhodopsin can now be incorporated into model membrane systems[25–27] with which it should be possible to study quantitatively the effects of each membrane component on membrane viscosity.

Rhodopsin collision frequency

X-ray evidence indicates that the average nearest-neighbour distance between rhodopsin molecules in the disk membrane is about 70 Å (refs 9 and 10). The average time τ, between collisions with another rhodopsin can be estimated with the following equation in which s is the distance rhodopsin travels between collisions:

$$s^2 = 4D\tau.$$

If the effective diameter of rhodopsin is about 45 Å (ref. 9), then s is 25 Å, and the average time between collisions is about 4 μs at 20°C. This is considerably less than the 20 μs relaxation time for rotational diffusion of rhodopsin, and indicates that the collision frequency between rhodopsin molecules is in the range 10^5–10^6 collisions per second.

The rapidity of rhodopsin-rhodopsin collisions points out an important consequence of the highly fluid nature of cell membranes, namely, the potential rapidity of reactions between membrane proteins. By partitioning into the membrane, protein molecules may achieve much higher effective concentrations and can in addition maintain favurable orientations as a result of protein-lipid interactions. Thus, since the viscosity for lateral motion in the membrane exceeds that of water by only a factor of 10–1,000, reaction rates both within and on the surface of a mebrane may potentially exceed by many orders of magnitude the rates which would occur if the reactants were dispersed throughout the aqueous phase.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Limits on variation of G from clusters of galaxies

If G decreases by a sufficient amount a cluster of particles will expand. In some cases the particles may have sufficient velocity to escape from the cluster as the gravitational binding decreases. Since it is known that clusters of galaxies and globular clusters currently exist with finite dimensions, limits can be set on the magnitude of the variation of G. In order to set a limit on the rate of change of the gravitational constant ($\dot{G}/G$), the dynamical effects on an isolated cluster of galaxies caused by non zero values of $\dot{G}/G$ were studied numerically. For a range of initial conditions, the maximum rate of change in G was determined which would still allow the existence of clusters of galaxies at the current epoch. A similar study was made for globular clusters and the results were found to be comparable.

The gravitational fields of both the cluster of galaxies and the globular cluster were simulated by Plummer potentials[1], and the effect on a particle (galaxy/star) of various rates of G variation were observed. The distribution of galaxies in a cluster at the time of formation is unknown, but if gravitational forces were dominant, a Plummer potential should provide a reasonable approximation to the cluster's field. The Plummer potential was initially designed to simulate the potential field of a globular cluster, and has the form

$$\Psi = \Psi_0/[1 + (r/r_0)^2]^{1/2}$$

where $\Psi_0 = M_0G/r_0$ and r_0 and M_0 are chosen using a method given by Henon[2]. We assumed that both the globular cluster and the cluster of galaxies were initially stable (twice the kinetic energy, T, plus the gravitational energy, Ω, is zero). For our standard cluster of galaxies we chose $M = 2.5 \times 10^{13} M_{\odot}$ and $r_0 = 0.17$ Mpc. This is about the mass of a Virgo type cluster, and r_0 was chosen a bit small to allow for expansion. It was demonstrated by Dearborn[3] that the resultant potential yields results consistent with those of an N-body calculation. Our standard globular cluster has $M = 10^6 M_{\odot}$ and $r_0 = 1.33$ pc. In reality, as the cluster expands due to a decreasing G, r_0 increases and $2T + \Omega$ becomes greater than zero. The shape of the Plummer potential would then change to a less stable configuration. In our calculation r_0 was held constant and $2T + \Omega$ kept equal to zero. Therefore, the potential was forced into a more stable shape than it would actually have, thus the limit obtained on G/G is even stronger than if we tried to vary r_0 for the potential with time.

The calculations were further simplified by using radial orbits for most of the work. For completeness, we did study several cases with circular orbits for both clusters of galaxies and globular clusters. Due to angular momentum effects, the expansion of circular orbits was even greater than that of radial orbits. Since orbits in real clusters are intermediate cases, the use of radial orbits gives the strongest limit.

For most of our calculations we used a form for G/G of

$$(\dot{G}/G) = -\alpha/t \quad \text{or} \quad G = G_i(t_i/t)^{\alpha}$$

when G_i is the value of G at time t_i.

This form is of particular interest, because it describes a change in G predicted by the Brans-Dicke theory. (The Dirac cosmology also predicts this form for G/G but with $\alpha = 1$). The t_i in this form is the time of the initial formation of galaxies in the cluster. This is not necessarily the time at which stars formed, but the time at which galaxy size masses condensed out of the intergalactic medium. Since such a time is open to speculations, we considered a range of initial times including 1.6×10^7 yr, 10^8 yr, and 10^9 yr. We feel, however, that the formation time given by Partridge and Peebles[4] of 2×10^8 yr (for a constant G) is reasonable. In fact, it seems reasonable that if G were initially larger, the speed of formation would be greater and t_i even smaller (B. Tinsley, private communication). For each of these initial times it was then possible to determine a limiting coefficient, α_{lim}, for which galaxies beginning near the centre of the potential attained escape velocity in a time less than the age of the universe.

Using relations given by Weinberg[5] it is possible to determine a limit on ω (the dimensionless coupling parameter in the Brans-Dicke Theory) for a given value of the cosmological deacceleration parameter, q_0, and the co-efficient α. The relations are:

$$\alpha/t_0 = 3q_0H_0/(2+\omega) \quad \text{for} \quad q_0 \ll 1$$

$$\text{and} \quad \alpha/t_0 = H_0/(2+\omega) \quad \text{for} \quad q_0 = 1/2$$

The present estimated value for q_0 is 1 ± 1 (ref. 6) but a recent study by Gott and Gunn[7] on the effects of infalling matter on a cluster of galaxies indicates $q_0 \ll 0.1$. Further arguments for a small q_0 are given from the evolution of Galaxies by Tinsley[8] and from cosmological deuterium production[9].

We also consider the form

$$\dot{G}/G = -1/\tau \quad \text{or} \quad G = G_i \exp\left(-(t-t_i)/\tau\right)$$

This exponential form for the variation of G/G is predicted by no theory known to us and is examined merely out of curiosity.

In Fig. 1 the maximum value of α (α_{lim}) consistent with the existence of clusters of galaxies is given for different initial times (t_i) and starting positions (R) in the cluster.

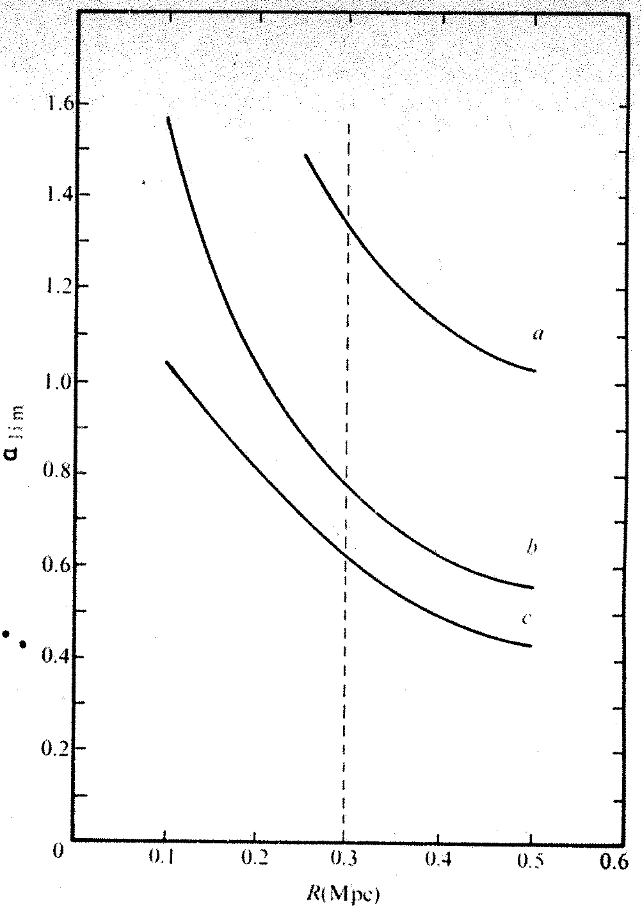

FIG. 1 Limiting α consistent with the existence of clusters at the current epoch for various initial radii, R and times t_i. Values of α_{lim} enable galaxies to escape in times less than the age of the universe. For $R < R_{\text{crit}}$ the Plummer potential is no longer accurate and α_{lim} is approximately constant due to the effects of close interactions. In our case R_{crit} is ~ 0.3 Mpc. Since α_{lim} increases for decreasing R the upper bound to α_{lim} is obtained at $R = R_{\text{crit}}$. $G/G_0 = -\alpha/t_0$. a, $t_1 = 10^9$; b, $t_1 = 10^8$; c, $t_1 = 1.6 \times 10^7$.

The limiting values of α were determined for a given t_i and R by evolving a case with several different αs. It was then possible to see at what value of α particles would escape in less than the age of the universe (escape was usually in less than 7×10^9 yr).

The initial position (R) of particles in the potential effects the limit that can be set on G/G. In agreement with N-body calculations[3], particles near the centre of the potential need a higher G/G to escape than do particles near the edge. But unlike the N-body solution, a particle at rest at the centre of a potential never escapes. In an N-body solution, such a particle would be perturbed into motion and escape along with other particles near the centre. It is therefore necessary to realize that the α_{lim} curves given in Fig. 1 (determined with a Plummer potential) should actually approach a finite value instead of going to infinity as the starting radius approaches zero.

It is possible to estimate a radius, R_{crit}, within which close interaction perturbations are significant relative to the background potential of the cluster. For initial radii, R, less than some radius, R_{crit}, the orbit of a particle in a Plummer potential is no longer a valid representation of the motion of a particle. Particles with $R < R_{\text{crit}}$ could be perturbed outward by close interactions, therefore the true value of α_{lim} does not increase significantly for $R < R_{\text{crit}}$. For an initial radius R less than $2\, r_0$ (0.34 Mpc in our case), the Plummer potential is probably no longer an accurate approximation to reality. Orbits for particles with initial radii ranging from

0.1 to 0.5 Mpc were studied, but we believe a value of $R_{\text{crit}} =$ 0.3 Mpc is a good lower bound to possible values. Since α_{lim} increases as R decreases the upper limit to α_{lim} occurs at $R = R_{\text{crit}}$.

Using $t_i = 2 \times 10^8$ yr and $R = 0.3$ Mpc, it is obvious from Fig. 1 that $\alpha_{\text{lim}} \cong 0.8$. This corresponds to values of $\dot{G}/G$ of

$$\begin{aligned}\dot{G}/G = -\alpha/t_0 &= 4 \times 10^{-11}\,\text{yr}^{-1} \text{ for } q_0 \ll 1\\ &= 6 \times 10^{-11}\,\text{yr}^{-1} \text{ for } q_0\\ &= 1/2 \text{ with } H_0 = 55\,\text{km s}^{-1}\,\text{Mpc}^{-1}.\end{aligned}$$

Even stronger limits might be achieved if different values for t_i and R are assumed. But the above estimates can be stated with reasonable confidence. (For the exponential case the limit was 10^{-10} yr^{-1}.) These limits on $\dot{G}/G$ are much stronger than Shapiro *et al.*'s[10] limit of 4×10^{-10} yr^{-1}. But the limit on the Brans-Dicke ω determined from these limits on α_{lim} is still very weak and does not put any real constraints on the scalar component in the Brans-Dicke theory. Therefore, the Brans-Dicke scalar component is not-inconsistent with the existence of clusters of galaxies. It can be said, however, that the Dirac Cosmology is not consistent with the present limit. Similarly the Hoyle-Narlikar Cosmology[11] with $\dot{G}/G \propto t^{-1}$ would seem inconsistant with the observations.

For the globular cluster case, a similar result can be obtained. But effects of $\dot{G}/G$ on a globular cluster will not be so dramatic because the crossing times are short relative to the time in which G changes significantly. The effect of $\alpha = 1$ on our standard globular cluster with $t_i = 1.6 \times 10^7$ yr was calculated. This t_i is appropriate for the time of formation of globular clusters[4]. After 1.5×10^{10} yr, a particle starting at $R = 1.5$ pc has an orbit 130 times larger. If the initial radius were reduced 130 times, the cluster evolves ~1,500 times faster and so would still expand to radii $\gg$1.5 pc. Initially, the cluster would be highly relativistic. Normally relativistic clusters collapse on time scales of 10^8 to 10^9 yr (ref. 12) but it is possible that a decreasing G may allow a relativistic cluster to expand to become a non-relativistic cluster instead of collapsing. Even so, such a cluster would have experienced a large number of stellar collisions. Such an extreme initial star density for any currently observed globular cluster seems very unlikely. So the limit on the variation of G from the current existence of globular clusters is approximately the same as that obtained from the current existence of clusters of galaxies.

While this work was in progress Morrison[13] proposed, by independent means, a limit of similar magnitude on $\dot{G}/G$. He used occultations timed on atomic time scales (IAT) to determine the slowing in the Earth's rotation due to tidal and nontidal ($\dot{G}/G$) forces. Previous determinations were based on data timed on a gravitational basis on which he assumed effects of $\dot{G}/G$ would not be apparent, leaving only tidal slowing. The close agreement of the decelerations rates then implies $\dot{G}/G = 0$ yr^{-1}, yielding an upper limit on $\dot{G}/G$ from the uncertainty in the data which is approximately the same as the limit estimated in this work.

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Radio spectra for three QSOs

RADIO spectra are presented for three sources of current interest: 1548 + 114 (4C 1150) which is one of two quasars separated by about 5 arc s which have been suggested as possibly associated despite widely disparate redshifts[1]; 1331 + 170 which is a very luminous QSO with a rich absorption spectrum and suggested line locking between the emission spectrum and two separate absorption systems[2]; and 1442 + 101 (OQ172) which has the currently highest known redshift[3], $z = 3.53$.

All three sources occur in two sections of the Molonglo catalogue survey currently being prepared for publication (J. M. Sutton, I. M. Davies, A. G. Little and H. S. M., unpublished). These cover declination strips approximately 2° wide, centred on +11.2° and +16.5° declination. The sources in this survey are being studied in detail. Optical identications have been carried out for a large fraction of the sources (C. Hazard and H. S. M., unpublished) and optical spectroscopic data on sixty-eight of the sources have been presented by Baldwin *et al*[4].

The radio spectra are given in Fig. 1. The flux densities at 80 MHz and 178 MHz for 1548 + 114 have been increased by 20% and 15% respectively[5,6]. The 606 MHz flux density was obtained at Arecibo and the 1,400 and 2,695 MHz flux densities have been obtained with the 300 foot telescope at Green Bank. The 5 GHz point for 1548 + 114 is from Pauliny-Toth and Kellerman[7] and that for 1331 + 170 has been obtained at Westerbork by Ekers (private communication). The spectrum of 1442 + 101 is taken from Murdoch and Hoskins[8] and further details are given there.

The source 1548 + 114 was first identified by Wills and Bolton[9] with a 17^{m} BSO. There is a second 19^{m} BSO 5 arc s following in right ascension and also a diffuse red object of about 19^{m} which may be a galaxy about 10 arc s preceding the bright object. Both BSOs have been confirmed as QSOs[1]. The optical positions measured with the Molonglo two coordinate measuring machine[10] to an accuracy 0.5 arc s in each coordinate for the stellar objects and 1 arc s for the g are:

17 max QSO	15 h 48 min 21.20 s	+ 11°29′47.0″
19 max QSO	15 h 48 min 21.52 s	+ 11°29′46.5″
19 mag g?	15 h 48 min 20.53 s	+ 11°29′45.1″

The 408 MHz position obtained from various Molonglo measurements including a recent measurement by J. M. Sutton is 15 h 48 min 21.12 ± 0.15 s + 11° 29′ 53 ± 3″. This is marginally consistent with the bright QSO but does not favour either of the other objects. A one dimensional brightness distribution at Westerbork at 5.0 GHz (Ekers, private communication) shows that about two-thirds of the

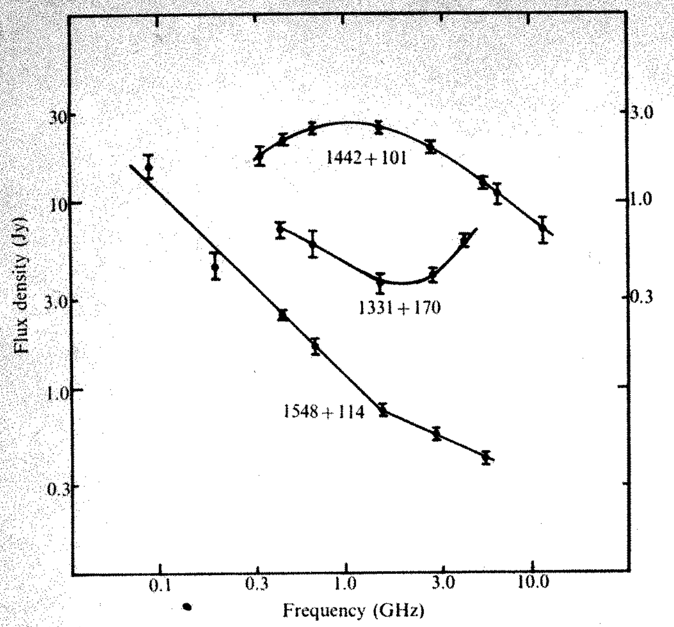

FIG. 1 Radio spectra of the three sources. The left flux density scale applies to 1548 + 114 and the right scale to 1442 + 101 and 1331 + 170. The flux density scale is in Jy. One Jansky = 10^{-26} W m^{-2} s^{-1}.

flux density is contained in an unresolved (<3 arc s) component which coincides with the bright QSO within one arc second in right ascension. There is also an extended component (~20 arc s) about 10 arc s preceding in RA.

The spectral index from 80 MHz to 1,400 MHz is 1.00 ± 0.05. From, 1,400 MHz to 5 GHz the spectral index is 0.47 ± 0.09. The two lines of fit are plotted in the figure but the change of slope must be more gradual than indicated. The best fitting single straight line over the range 80 to 5,000 MHz represents a spectral index of 0.80 but is a poor fit (χ^2 probability ~5 × 10^{-5}). The spectrum is therefore of the centimetre excess (CE) type suggesting the existence of a compact component.

All of the observations used in plotting the spectrum were obtained with beams of some minutes of arc. The 408 MHz position suggests that most of the low frequency flux density comes from the bright QSO. Since the high resolution Westerbork data suggest the same at high frequency, the location of the CE component is not clear. It seems unlikely to be the extended component. The structure is probably more complex than the two components so far revealed.

The source 1331 + 170 has a strong centimetre excess suggesting a very compact component. The optical position is 13 h 31 min 10.00 s, +17° 04′ 26′.0″.

The source 1442 + 101 is a QSO with a redshift of 3.53. Although the source peaks in the vicinity of 1 GHz there seems to be no evidence of centimetre excess emission up to 10 GHz. Of course, as a result of the high redshift, the peak emission in the rest frame of the source is close to 5 GHz.

By contrast, however, the secondary peak for 1331 + 170 (z = 2.08) must be greater than 15 GHz in the emission frame. Both 1331 + 170 and 1442 + 101 are reported to have rich absorption spectra, and both are highly luminous objects. Their radio spectra are consistent with the claim by Setti and Woltjer[11] that this region of the M-z plane is occupied only by sources with anomalous radio spectra.

The optical positions were measured on the two-coordinate measuring machine by Miss Sue Howard. I thank Dr R. D. Ekers and Dr E. J. Wampler for communicating their results prior to publication. The Molonglo Radio Observatory is supported by the Australian Research Grants Committee, the Sydney University Research Grants Committee and the Science Foundation for Physics within the University of Sydney. The National Astronomy and Ionosphere Center and the National Radio Astronomy Center are thanked for observing facilities at Arecibo and Green Bank respectively. The National Radio Astronomy Observatory is operated by Associated University Inc. under contract to the National Science Foundation.

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Correlation search for dispersed radio emission from the galactic centre

WE report here the results of a search for fluctuations in the intensity of radio emission from the vicinity of the galactic centre using a two-channel cross-correlation technique.

The initial incentive to look for a time variable radio flux from the region of the galactic centre arose primarily[1] from the reports[2,3] of impulsive gravitational radiation. Searches for impulsive radio events have been reported recently[1,4–7]. These observations have been restricted by the necessity to recognise individual pulses in the presence of fluctuation noise. They refer therefore to pulses substantially greater than the r.m.s noise level. For example, Hughes and Retallack[4] report pulses of height ≥ 85 f.u. at 858 MHz occurring at a rate of ≈ 10^{-4} s^{-1}. Other observers[1,5,6] have utilized the expected delay in pulse arrival times at different frequencies due to velocity dispersion in the interstellar medium in order to reject impulsive noise of local origin.

We utilize the dispersion phenomenon in our search by looking for a delayed, correlated component to the intensity fluctuations at two neighbouring frequencies. Unlike those mentioned above, this method allows the detection of dispersed transients well below the r.m.s. fluctuation noise level. The technique is therefore particularly suited to the detection of low level signals, distinguishable only by their dispersed character. We describe its use in a search for dispersed noise having an arbitrary probability density function, for example pulses with amplitudes below those already reported[4]. The results of a concurrent examination of the data for individual large pulses will be reported elsewhere.

If $x(t)$ and $y(t)$ are the envelope detected outputs of the two telescope channels, their time lagged cross product is $R_{xy}(\tau) = \langle x(t)\cdot y(t+\tau)\rangle$, averaged over the observing time interval. The values of this mean lagged product are the cross-correlation estimates. Plotted as a function of the time lag, τ, they constitute a correlogram which may be examined for evidence of a dispersed fluctuation noise component. Provided the characteristic time scale of the fluctuations is shorter than the time delay due to dispersion in the interstellar medium, a characteristic source signature, distinguishable from local noise, should appear in the correlogram. For non-periodic signals, a random pulse train for example, this signature takes the form of a positive peak in $R_{xy}(\tau)$ at a time lag, $\tau = \Delta t$, the time delay expected between channels separated in frequency by $\Delta\nu$(MHz). Δt(s) $= 8 \times 10^3 DM(\Delta\nu/\nu^3)$ where DM is the dispersion measure of the variable source.

The height of the peak will be proportional to the mean square value of the correlated flux density variations. Its width indicates their time scale. The peak amplitude may be depressed and the characteristic width may be increased by: (1) dispersion within the non-zero channel bandwidths; (2) a non unique value of dispersion measure; (3) post-detector filtering; (4) decorrelation between the signals occurring at the source or in the medium.

The signal to r.m.s. noise ratio of a correlation estimate, $R_{xy}(\tau)$ increases as the square root of the product of the channel bandwidth and observing time. This indicates the high sensitivity of the correlation method for long observing times and wide bandwidths. In practice the presence of slow correlated instrumental drifts reduces the sensitivity below that expected.

The 150 m meridian transit telescope[8] of the University of Otago, situated at the Invermay Radio Observatory near Dunedin, New Zealand (46°S) was used in the search. The telescope was operated at two frequencies, 120 MHz and 130 MHz as a two element phase switched interferometer in order to minimise interference and reduce instrumental drift. The effective aperture of the telescope is 5×10^2 m^2. The telescope was steered to declination $\delta = 29.0$°S for the two months search duration, from June through July 1973. The area of the search was a (2° × 8°) strip centred on the galactic centre covering essentially the same region as that covered by Hughes and Retallack[4]. It included both Sgr A and the source they reported at α(1950) = 17h 48.2 min, δ(1950) = −28° 58′. The duration of transit between the half power points of the antenna beam was approximately 50 min. The system temperature reached a maximum of 2,200 K during the transit of the galactic centre. The r.m.s. temperature fluctuation was typically 1 K (6 f.u.) for a post detector integration time constant of 10 s. Calibration was performed periodically by noise injection at the antenna.

The receiver outputs, following envelope detection were low pass filtered with time constant 10 s, this value being approximately the pulse broadening time within the receiver bandwidth of 1 MHz expected for a hypothetical impulsive source having a dispersion measure of 2×10^3 cm^{-3}pc. The integrated signals were then AC coupled with time constant of 100 s (in order to reduce slow drifts) to the data logging system. This consisted of a dual channel chart recorder used for visual detection of dispersed events and two dual channel magnetic tape recorders on which the signals were written in FM mode together with a stable clock reference signal used for synchronizing the tape readout. The data were sampled at 0.30 Hz. Cross correlation analyses were performed on 30 min data blocks.

For the purposes of envelope correlation only those records of highest quality were used. Any 30 min blocks which contained non dispersed individual pulses greater than about 20 f.u. were rejected from this analysis. Twenty-four individual correlograms, representing 12 h (20% of all data) were computed for delay times, τ from −400 s to +400 s. A delay of $\tau = +400$ s corresponds to $DM = 10^4$ pc cm^{-3}. Figure 1 shows the computed average of the twenty-four correlograms. The most noticeable feature is the positive peak at zero delay. This peak, 10^{-2} square degrees (4×10^{-1}(f.u.)^2) in height indicates the presence, in spite of careful selection of data, of undispersed system temperature fluctuations with an r.m.s. value of 10^{-1}K. For time lags $|\tau| \gtrsim 20$ s ($DM \geq 500$ pc-cm^{-3}) the cross covariance fluctuations have an r.m.s. value of 0.1(f.u.)^2. Any dispersed noise component with variance exceeding 2×10^{-2}(K)^2 corresponding to 0.8(f.u.)^2 for a source near the galactic centre, would be clearly visible.

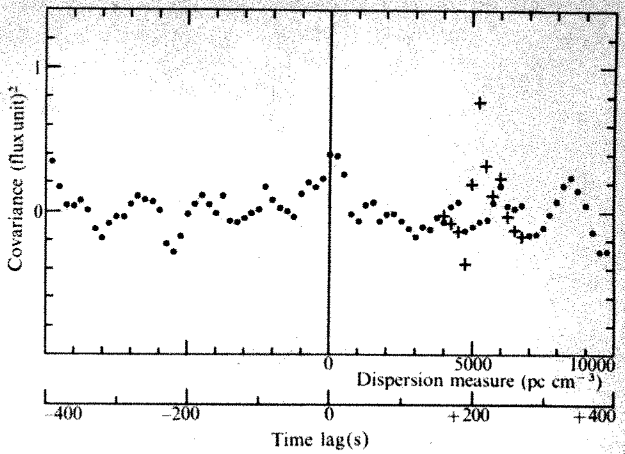

FIG. 1 Average cross-correlogram of galactic centre radio wave intensity fluctuations at frequencies of 120 and 130 MHz. Crosses indicate the signature due to a random sequence of dispersed ($DM = 5 \times 10^3$ pc cm^{-3}) calibration pulses of variance 1.2(f.u.)^2.

To illustrate this and to check the computation procedures, a random sequence of dispersed pulses was generated, added to the raw data and processed. The resulting correlogram signature shown in Fig. 1 is consistent in location ($DM = 5 \times 10^3$), shape (full width at half maximum = 20 s) and covariance (0.8(f.u.)^2) with that expected for these facsimile pulses. The mean pulse rate, $\alpha = 2.5 \times 10^{-2}$s^{-1}, and the amplitude of each pulse was $s = 1.5$ f.u., approximately ¼ the r.m.s. noise level.

We conclude that a conservative upper limit of 0.8(f.u.)^2 may be assigned to the mean square flux density variations at 125 MHz of a variable source located either at the position of the impulsive source previously reported[4] or in the direction of the galactic centre. This upper limit remains less than about 3(f.u.)^2 for sources with δ(1950) = 29.0 ± 1.0°S, and α(1950) = 17 h 44 min ± 18 min. In order to attach a physical significance to our results and to relate them to other observations, we consider several sources of variability.

As a possible scenario we assume the previously reported pulses[4] to be the chance superposition of more frequent, randomly occurring subpulses generated perhaps in stellar events in the region of the galactic centre. It is easy to calculate the variance $\sigma^2(s) = \langle s^2\rangle - \langle s\rangle^2 = \alpha e^2/T$ and the mean flux density $\langle s\rangle = \alpha e$ for impulse, e, and resolving time, T. If the 'giant' pulses reported contain m unresolved subpulses in time T we may assume for illustrative purposes that, as reported $me = 8.5 \times 10^{-25}$J m^{-2} Hz^{-1} (85 flux unit seconds), $T \sim 1$ s, and the 'm-fold' pulse rate $\alpha P(m-1, T) \approx 10^{-4}$s^{-1} where $P(m-1, T)$ is the Poisson probability of observing $(m-1)$ pulses in time interval T. For $m = 3$, $\alpha \approx 6 \times 10^{-2}$s^{-1}, and $\sigma^2 \approx 50/T$ (f.u.)^2. A value of $T \approx 20$ s is appropriate to our observations at 125 MHz, taking into account post detector integration and convolution with the receiver bandwidths of dispersed impulses generated by a source or sources having $DM \leq 5 \times 10^3$ pc cm^{-3}. The amplitude of the correlogram signature would in this case be $\approx$2.5 (f.u.)^2, more than twice the amplitude of the facsimile signature shown in Fig. 1

and ≈25 times the r.m.s. correlogram noise. Other values of m do not significantly affect this result which clearly shows the 'giant' pulse model to be unlikely. Raising m, will in the limit, simulate a physical situation in which gaussian noise fluctuations are generated at the source. Our results applied, say to Sagittarius A, place a conservative upper limit to its correlated variance over a 10 MHz band centred at 125 MHz of $\sim 1(\text{f.u.})^2$ for periods from 50 to 500 s. This upper limit to the correlated variance of $1(\text{f.u.})^2$ may also be used to place a limit on the flare star contribution[9] to the time averaged brightness temperature of the galactic centre region. If the flare stars are concentrated in a 2 $(\text{deg})^2$ region of thickness $\Delta\ (DM) \approx 10^3$ pc cm^{-3}, a realistic distribution[10] of flare event sizes then gives $\langle T_{FS}\rangle \leq 5$ K. This is a fractional contribution of $<10^{-3}$ to the mean temperature of the region.

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Near Infrared Observations of Cygnus X-3

THE system known as Cygnus X-3 has been extensively studied at radio wavelengths (where it exhibits extraordinary variability[1-3]), in X rays (where it exhibits a 4.8 h period[4-6]), and in the infrared at 1.6 and 2.2 μm (where it also displays a 4.8 h period as well as large flares[7,8]). Attempts have also been made to detect Cyg X-3 in visual and near infrared wavelengths[9,10]. Here we present the results of a series of observations made at near infrared wavelengths, $\lambda_{\text{effective}} \simeq 0.85$ μm on ten nights in July and August 1973.

Our observations were made with a magnetically focused image tube, ITT F 4089, which has an S-25 photocathode that is sensitive in the near infrared. The image tube was placed behind a Wr-88A filter and the exposures were recorded on Kodak 103a D plates pressed against the output P-20 phosphor. Typical exposure times were 15 min at the 1-m reflector telescope of the Wise Observatory. This equipment is somewhat more sensitive in the near infrared than previous systems[10] used for this problem and our coverage is much more extensive.

No object was detected to our plate limit at the position of the radio source Cygnus X-3. Our lower limits for the magnitudes at λ_{eff} vary from $I \sim 17.5$ mag to 18 mag depending on conditions; observations were made at the following universal times: July 3.95, 4.99, 6.97, 7.92, 8.83, 9.85 and August 1.03, 1.87, 3.98 and 6.90. The magnitude limits given above are crude estimates based upon plates, taken with the same equipment, of M15 (Sandage[11] has reported accurate *UBV* photometry for M15) and the relationship between *B*, *V* and *I* colours given by Johnson[12]. It should be noted that the 4.8 h periodicity of Cyg X-3 was studied on July 9.2–9.55 by Becklin *et al.* (ref 8) at 1.6 μm and 2.2 μm (with the Hale 200-inch telescope) and at X-ray wavelengths (with the Copernicus satellite. Their observations took place between two of our exposures listed above. It should also be noted that a radio event apparently occurred in Cyg X-3 near the beginning of our observation period in July[13] and our results therefore limit the near infrared strength of this event.

Our upper limits on the near infrared radiation from Cyg X-3 can be compared with expectations based on simple models. The expected *I* magnitude ($\lambda_{\text{eff}} = 0.9$ μm) is

$$m_I = m_K + E_{I-K} + (M_I - M_K)$$

where[7] $m_K = 11.4$ mag, E_{I-K} is the selective absorption, and $(M_I - M_K)$ is the difference in absolute magnitudes between $\lambda = 0.9$ μm and 2.2 μm. The selective absorption can be estimated to be $E_{I-K} \simeq 6$ mag by using the obvious relation $E_{I-K} = (E_{H-K})(E_{I-K}/E_{H-K})$, the observational determination that[7] $E_{H-K} \simeq 1.22 \pm 0.1$ mag and the average ($\simeq 4.9$) of four separate determinations of E_{I-K}/E_{H-K} (4.25 in the direction of Aquilla-Cygnus[14], 5.43 to the Galactic centre[15] and 4.48 and 5.30 from measurements involving M supergiants[16]). For a typical early-type supergiant such as has been associated with a number of other X-ray sources, one has $M_I - M_K = -0.5$ mag. Thus a typical value to have expected for m_I would have been $m_I \sim 16.9$ mag (with more than 0.5 mag uncertainty due to the imprecisely estimated selective absorption). Our results are consistent with, but do not completely establish because of uncertainties in selective absorption and plate limits, a spectrum that is flatter than the $f_\nu \sim \nu^2$ associated with a supergiant in this wavelength region. It will be important to attempt to obtain in the next observing season a better determination of the selective absorption to Cyg X-3 in order to establish firm limits on the shape of the spectrum.

The expected visual magnitude for Cyg X-3 is $m_V = 24.8$ mag if one uses results in the direction of Aquilla–Cygnus[14] or the Galactic centre[15] to calculate the selective absorption and $m_V = 28.5$ mag if one uses the average result obtained with M-supergiants[16]. In both cases, we have assumed $M_V - M_K = -0.9$ mag corresponding to an early O or B star[14]. If we had assumed a flatter spectrum, we would of course have obtained fainter values of m_V, for example, ~1 mag fainter for an AO star. The estimated values for m_V are all consistent with the limit of $m_V = 23.9$ mag set by Westphal *et al.*[9]. The estimated total visual absorption varies between $A_V = 18.3$ mag to 20.4 mag, depending on how one calculates the selective absorption.

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Extended Glow Preceding Reappearance of Saturn during a Lunar Occultation

THE recent occultation of Saturn by the Moon[1], visible across Canada in the early morning of Wednesday, October 17, 1973 provided the opportunity to observe and photograph the reappearance of the planet from behind the dark lunar limb. Teams of observers from the Calgary Centre of the Royal Astrononomical Society of Canada and the University of Calgary Physics Department established telescopes at various sites in Alberta but poor weather restricted photography of this event to times rather late in the reappearance and to a "close approach" sequence at the University of Calgary Rothney Astrophysical Observatory south of the shadow path.

The present observation was made from the northernmost site at Ardrossan, near Edmonton, Alberta (53° 33′ 45″ N, 113° 07′ 50″ W) where a team of four observers were spaced along a line at right angles to the shadow path several hundred metres apart in an effort to observe photoelectrically as well as visually the grazing occultation of the 8 mag Titan by the south lunar limb. This attempt was thwarted by instrument problems and by intermittent clouds, but the sky cleared considerably by the predicted time of Saturn reappearance with only light haze surrounding the 66% sunlit Moon. Cameras were mounted on three of the four telescopes at this site and observers were linked by intercom to each other and to a tape recorder on which WWV time signals as well as observer comments were recorded. The fourth observer was to provide careful visual observations and detailed timing of the reappearance and was equipped with a 6 inch *f*/9 Newtonian telescope equipped with a 12 mm eyepiece and a ×2 Barlow lens (overall magnification ×230) to ensure that light from the bright limb did not obscure the observation in the 12 arc min field of view at the moment of egress.

The observation upon which this report is based was made by the visual observer at a time between 1 s and 2 s before the appearance at the dark lunar limb of the first pin-point of light from Saturn's rings. At this time, the observer's eye was attracted to a faint glow which he probably first saw by averted vision but which delineated the dark limb and which appeared like seeing "a campfire on the other side of a treeless hill on a dark night". The extent of the glow was estimated afterwards to have been a little greater than the east–west extent of the rings. The pin-point of light from the rings which appeared in the centre of the faint glow rapidly brightened and became bigger as the rings became visible following the third contact. The glow increased slightly in width and showed easily detectable Moon limb curvature on its sharp edge but at about 4 s beyond third contact the glow was indistinguishable against the increasing ring brilliance.

Superficial explanations for this glow can be quickly dismissed. If internal reflection within the telescope of light from the bright lunar surface had caused the glow, it was certainly a coincidence that it not only appeared in the exact position where the rings showed themselves but also was aligned with the direction and position of the dark Moon edge. Mist in the Earth's atmosphere could have produced a hazy glow just as the first light of the rings appeared but this would almost certainly have been circular from the initially observed edge of the rings. This must remain a distinct possibility but it is at least as tempting to associate this glow with the planet and a tenuous outer atmosphere surrounding Saturn and its rings which is normally invisible against the planet's brightness. Observation of a faint outer ring (the so-called D ring) has been referred to several times this century (see for example, Guerin[2]) but not confirmed by modern photography. These observations may be related to the present result. Furthermore a very similar observation to that seen for Saturn was reported by Brock[3] for the planet Jupiter when it was occulted by the south-limb mountains of the Moon. At that time a glow was noted to be moving along above the mountain range during the grazing occultation. This glow associated with Jupiter was reported to vanish immediately the planet appeared and it was not seen by observers situated one quarter of a mile north and south of the observer.

Thus, a tentative conclusion for these unique visual observations must be that these giant planets have extended atmospheres associated with them, an idea suggested for Jupiter by Peek[4] who postulated an upper atmosphere consisting of light elements and extending to great heights above the cloud layers. Estimates of total pressure of gas above the cloudtops from spectroscopic data vary from 2 to about 13 atm (ref. 5). Such gaseous atmospheres would not be expected to scatter sunlight but could presumably emit light equivalent to airglow or even auroral luminosity under the action of the solar wind. The existence of a magnetosphere and Van Allen belts is inferred from the intense decimetric radio bursts detected from Jupiter[6]. The requisite particle flux for excitation of such luminosity is thus present, at least for Jupiter. Radio bursts from Saturn are much weaker but the similarity of this planet to Jupiter would lead one to believe that such luminosity is possible from Saturn also.

In the case of the present observation of Saturn, if we assume 1.5 s for the time between appearance of the glow and that of the rings, the extension beyond the rings is approximately 5,000 km or 4% of the planet's diameter. The observer's reports of the glow accompanying Jupiter would indicate a glow extending about 1,000 km above the cloud tops of Jupiter, or less than 1% of the planet's diameter. The geometry of these events was such that the glow was observed close to the polar regions of both planets.

It is obvious that repeated measurements of this phenomenon, particularly careful photoelectric observations, are necessary in order to verify the existence of the glows but these can be made only very infrequently. Of much greater importance, bearing in mind the approach of the Pioneer spacecraft to Jupiter, is that extended glows should be searched for in data from these deep space probes.

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Interpretation of Solar Hard X-ray Burst Polarisation Measurements

Tindo *et al.* (refs 1 to 3 and unpublished results) have reported the first observations of polarisation of solar flare emission in the hard X-ray band (around 15 keV) and have made a preliminary interpretation of these[3]. Their results depend, however, on the removal of systematic differences in sensitivity of the detectors used in the Intercosmos Thomson-scattering polarimeters, by calibration on an 'unpolarised' source. We point out here that the results as presented by Tindo *et al.* are in fact in error since their calibration was based on the assumption that the hard X-ray flux from the flare itself tends to zero polarisation in its final stages of decay. This assumption is invalidated by the presence, in the total flux, of a large polarised contribution from photospheric albedo photons[4,5]. We further consider how much this calibration error may affect interpretation of the Intercosmos results in terms of flare particles and suggest how further theoretical work, combined with results from a laboratory calibrated polarimeter, can yield information on the spatial location of hard X-ray flares as well as on their true polarisation.

Hard X-ray polarisation measurements have a key role to play in the formulation of flare theories since the degree and plane of polarisation indicate the velocity distribution of the bremsstrahlung source electrons—in particular, whether they stream along the flare field lines or are magnetically trapped—thus giving insight into the flare particle acceleration mechanism[3,6,7]. Yet more important, future observations of the variation of the polarisation with photon energy will provide a means of determining the photon energy above which non-thermal electrons rather than the multi-temperature flare plasma is the dominant X-ray production mechanism[8]. This question is fundamental to the overall energetics of a flare due to the characteristically steep electron spectra involved. Thus if the thermal component dominates to about 30 keV, the electron streams responsible for the emission above this energy would carry only a small proportion of the total flare energy and so be a minor artefact of the release process. On the other hand, if non-thermal emission dominates to below 10 keV, these streams carry quite adequate energy to supply the entire thermal flare and so may be fundamental to flare heating[9].

In the two main models of hard X-ray flares, the primary bremsstrahlung flux is partially linearly polarised either along or across the solar disk radius through the source[3,6,7] and so may be expressed in terms of its components $F_1^{\parallel}$ and $F_1^{\perp}$ in these respective directions. Tomblin[4] and Santangelo *et al.*[5] have, however, emphasised the importance of photons backscattered from the photosphere which, in the deka-keV range, may be reflected with efficiencies up to 80%. Santangelo *et al.*[5] have further pointed out that this albedo contribution will be polarised by the scattering, so modifying the polarisation of the total X-ray flux observed and even introducing polarisation[8] into the flux originating in unpolarised primary emission such as a multi-thermal source[8] or a non-thermal one in which the velocities are randomised. The degree of this scattering polarisation has so far only been calculated[10] in the case of low photon energies where it is $\lesssim 2\%$. At these energies, however, the albedo contribution to the total flux is itself small due to photo-electric absorption[4,5]. In the deka-keV range the process is much nearer to pure scattering for which the polarisation in the scattered component itself may approach 100% for 90° scattering, a figure not much reduced even for multiple scattering[12] as involved here. (This situation is very similar to the optical polarisation of the blue sky[11,12].) The plane of maximum intensity in this scattering polarisation will always lie transverse to the solar radius through the source[5,8]. Thus if the photospheric albedos are $\rho^{\parallel}$ and $\rho^{\perp}$ respectively for radiation polarised in the radial and transverse directions (depending on the photon energy and the scattering geometry), the secondary (reflected) contributions to the observed total fluxes are

$$F_2^{\parallel} = D^{\parallel}\rho^{\parallel}F_1^{\parallel} \qquad (1)$$

and

$$F_2^{\perp} = D^{\perp}\rho^{\perp}F_1^{\perp}$$

Here $D^{\parallel}$ and $D^{\perp}$ measure the directivity of the primary source (for the respective polarisation planes) in the direction of the photospheric scattering centre as compared to the Earth direction. (Inclusion of these two parameters is essential since any non-zero primary source polarisation will in general be accompanied by a source directivity and a variation of the polarisation with direction[6,7]—that is, $D^{\parallel} \neq D^{\perp} \neq 1$ except in special cases.) Total observed fluxes in the two planes are therefore

$$F_T^{\parallel} = F_1^{\parallel}(1 + \rho^{\parallel}D^{\parallel}) \qquad (2)$$

and

$$F_{\perp}^{\perp} = F_1^{\perp}(1 + \rho^{\perp}D^{\perp})$$

where $\rho^{\parallel}$ and $\rho^{\perp}$ may be taken as constant through the burst while $D^{\parallel}$ and $D^{\perp}$, as well as $F_1^{\parallel}$ and $F_1^{\perp}$, will vary during its development.

The physical basis of the calibration method of Tindo *et al.* (refs 1 to 3 and unpublished results) is that, late in the burst, source electron velocities become randomised so that the intrinsic polarisation tends to zero ($F_1^{\parallel} = F_1^{\perp}$). (This also implies $D^{\perp} = D^{\parallel} = 1$.) This is reasonable but, in applying it, Tindo *et al.* have neglected the albedo contributions in equation (2) which are present during calibration (as at all times); that is, they have taken $F_T^{\parallel} = F_T^{\perp}$ and attributed the imbalance in their detector count rates entirely to differing sensitivities. Thus, if we suppose for the present purpose that the calibration was done in terms of correction of the radial (∥) count rate relative to the transverse (⊥), then all the transverse count rates from the experiment have been reduced by a calibration factor

$$1/\beta^{*} = 1/\beta\gamma \quad \text{where } \gamma = (1 + \rho^{\perp})/(1 + \rho^{\parallel}) \qquad (3)$$

instead of by $1/\beta$ where β is the true transverse/radial counter sensitivity ratio. Since, for 90° scattering, $\rho^{\perp}$ may tend to 1 and $\rho^{\parallel}$ to 0, γ may be as high as 2, implying a serious discrepancy between the polarisations deduced by Tindo *et al.*[3] and those actually present in the total flux from the flare. In the late decay phase, for example, the polarisation in the observed flux due to scattering would amount to

$$p_s = (F_T^{\perp} - F_T^{\parallel})/(F_T^{\perp} + F_T^{\parallel}) = (\rho^{\perp} - \rho^{\parallel})/(2 + \rho^{\perp} + \rho^{\parallel})$$

which could be as high as 1/3 (for 90° scattering) rather than the zero value attributed in the experiment calibration.

The basic aim of the Intercosmos experiments was to determine the polarisation characteristics of the primary X-ray burst source. Correction of the results of Tindo *et al.*[3] for albedo effects will require further theoretical work on the scattering process, but some tentative conclusions may be drawn regarding the extent to which their results deviate from this intrinsic polarisation. This latter may be written as $P_1=(r_1-1)/(r_1+1)$, where $r_1=F_1^{\perp}/F_1^{\parallel}$, whereas the false polarisation deduced by Tindo *et al.* is $P_0=(r_0-1)/(r_0+1)$ where r_0 is related to r_1 by equations (2) and (3), namely

$$r_0=r_1(1+\rho^{\parallel}D^{\parallel})/(1+\rho^{\parallel})\times(1+\rho^{\perp})/(1+\rho^{\perp}D^{\perp}) \qquad (4)$$

First, it is interesting to note that if source directivity were neglected in equation (4), then $D^{\parallel}=D^{\perp}=1$ and $r_0=r_1$ so that the results presented by Tindo *et al.*[3] would indeed represent the primary source polarisation, though not the polarisation actually present in the total flux observed. This coincidence arises because the fractional albedo contribution would remain the same, in this case, between calibration and the burst itself. In practice, however, neither the polarisation nor the intensity of the primary emission will be the same in the Earth and photospheric directions, except by special coincidence. As a realistic example of primary emission characteristics we may consider the thick target model (analysed by Brown[7]) in which an electron stream bombards the low chromosphere. For a flare at CMD = 45° (90° scattering), this model predicts $r_1\simeq0.6$ and $D\simeq4.8$ for 15 keV photons, giving $r_0=0.21$ by equation (4) (with $\rho^{\perp}=1$, $\rho^{\parallel}=0$). The intrinsic source polarisation is thus $P_1=-0.25$ as against the value $P_0=-0.5$ which would have been deduced by Tindo *et al.* in this idealised case. For different scattering geometries and photon energies it is quite feasible that neglect of the albedo contribution might result in deduction of the incorrect plane of maximum intensity as well as degree of polarisation.

In conclusion it is clear that no definitive interpretation of hard X-ray flare polarisation measurements may be made without full theoretical investigation of $\rho^{\parallel}$ and $\rho^{\perp}$ which depend on the scattering geometry, and so on the unknown location of the primary source in the solar atmosphere. If, however, future experiments are conducted using laboratory-calibrated detectors then the polarisation observed late in the flare can be attributed to photospheric scattering and, by comparison with theory, could be a valuable tool in inferring the primary source location without direct angular resolution. Once this is known, the albedo contribution may be subtracted from observations during the burst itself and the polarisation of the primary source deduced.

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Meteor of August 10, 1972

AN extraordinarily large and brilliant meteor was seen by many observers[1] in the western United States and Canada at 2030 UT (1430 local time), on August 10, 1972. A satellite-borne near infrared radiometer also detected and tracked it. W. T. Rogers, a surveyor of Billings, Montana, obtained a ground based sighting which provided additional altitude information. Using his measurement and the satellite data we calculated the trajectory.

The meteor first became sufficiently hot to be detected over Utah and it finally cooled below the detection threshold over Alberta, 100 s and 1,500 km later[2]. Its path is shown in Fig. 1. The meteor very narrowly missed hitting the Earth, its perigee being only 58 km. It apparently did not fragment. Quantitative trajectory parameters are given in the table. Because of the availability of the satellite data the accuracy of this trajectory far exceeds that determined for any other meteor.

Table 1 Meteor Trajectory

Observation	GMT (s)	Velocity (km s^{-1})	Geodetic altitude (km)	Geodetic latitude north	Geodetic longitude west
Initial	73708.9	15.03	75.98	38.389	112.575
Perigee	73747.5	14.60	57.85	44.386	112.993
Final	73810.1	14.21	101.55	53.331	113.785

We obtained estimates of the mass, kinetic energy and size, using the velocities from Table 1 and the measured infrared radiation. We equated the loss in kinetic energy to the thermal radiation. This neglects the small fraction of the energy converted into acoustic waves. We calculated the integrated

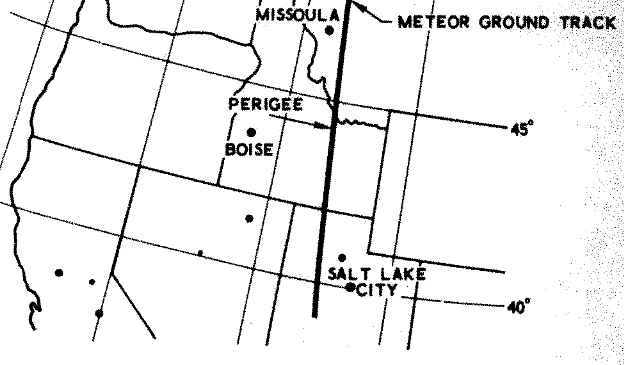

Fig. 1 Path of meteor of August 10, 1972.

thermal radiation from that within the infrared passband assuming that the source could be approximated by a 3,000 K blackbody. We believe that the resultant thermal energy is accurate to within a factor of four, with most of the error arising from the temperature and blackbody assumptions. The mass turned out to be 10^9 g or about 2% of the estimated mass of the Barringer meteor which made the Arizona Crater. The calculated equivalent diameter is 4 m, assuming the density of iron. The initial kinetic energy was 10^{14} J, or approximately the yield of the nuclear weapons which destroyed Hiroshima and Nagasaki. The meteor approached the Earth at 15 km s^{-1} and if it had been at a slightly lower altitude, the damage would have been very extensive. Fortunately meteors of this size are exceedingly rare.

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Aftershocks Caused by the Novaya Zemlya Explosion on October 27, 1973

IN the autumn of 1973 seismic signals were recorded from three underground nuclear explosions on Novaya Zemlya in the Arctic Ocean. Two of these explosions were located at a new site on the southwestern part of Novaya Zemlya. The explosion on October 27 at the new site was followed by a suit of 10 small events, all of which occurred within 4 h of the explosion. Here we give the locations of the explosions and the afterevents based on data from Nordic and North American stations and the discrimination results based on shortperiod data obtained at the Hagfors Observatory[1] (HFS) in Sweden.

Table 1 summarises pertinent location data with their error estimates for the ten afterevents, the 1973 explosions and an explosion in 1969 used in the discrimination study. The epicentres are obtained by a joint epicentre location technique[2] utilising arrival times of seismic P and S phases. The location errors given as standard deviations include error estimates of the traveltime model and should thus be regarded as fairly realistic estimates of the actual errors. Arrival time data reported by Nordic and North American stations are used. Epicentre data for two explosions reported by NOAA[3] are also given in Table 1. The explosions on September 27 and October 27 are both located about 200 km south of the main test site of Novaya Zemlya, where the explosion on September 12 was fired. The map (Fig. 1) shows the locations of the main test site close to the channel between the northern and the southern island and the new test site in the southwestern part of the island.

Figure 2 displays the shortperiod P-wave signals recorded at HFS from the explosions in 1973 and seven of the afterevents. No surface waves for the afterevents are detected at HFS. The body wave magnitudes, m_b (HFS), which are supplemented in Table 1, range over four orders of magnitudes. Four magnitudes correspond to a dynamic range of 80 dB which puts rather strong requirements on the recording equipment. To illustrate the dynamic range of seismic signals, the signals from the three explosions, covering a range of two magnitudes or 40 dB, are plotted in the same scale. The afterevents are plotted with a gain of roughly 500 times that of the explosions. Figure 2 shows the simple, pulselike waveforms of the explosions[4] compared with the more complex afterevent signals

To study the differences of the P-signal spectral content between the explosions and the afterevents the third moment of frequency (TMF) parameter, which is a measure of the high frequency content of the signals, is calculated for the signals recorded at HFS. TMF has previously been used to discriminate between explosions and earthquakes[5,6]. The TMF values are given in Table 1. The high frequency content is, however, not only a function of the source type but also of the magnitude of the event. This magnitude effect is clearly demonstrated in Fig. 3 where TMF is plotted against m_b (HFS). The trend for the Novaya Zemlya explosions covering two orders of magnitudes is very clear. Extrapolating this trend down to magnitudes comparable with those of the afterevents would yield a very large difference between the two types of events. This difference indicates that the explosions and the afterevents are different types of seismic sources.

As far as we know no seismic activity has been reported in the Novaya Zemlya region nor has any of the earlier explosions been followed by aftershocks. Aftershock activities have however been reported following high yield explosions on the Aleutian Islands[7] and at the Nevada Test Site[8,9]. The events observed in these regions are of two different kinds. One type is associated with the deterioration of the cavity and these events are located in the immediate vicinity of the cavity. Such events have been observed soon after the explosion and they continue until the cavity completely collapses. The second type of events is the explosion stimulated tectonic events. These may occur at distances greater than 10 km from the explosions and such events have been observed months after the explosions. The tectonic events seem generally to be weaker than those related to the cavity deterioration. The numbers of after-

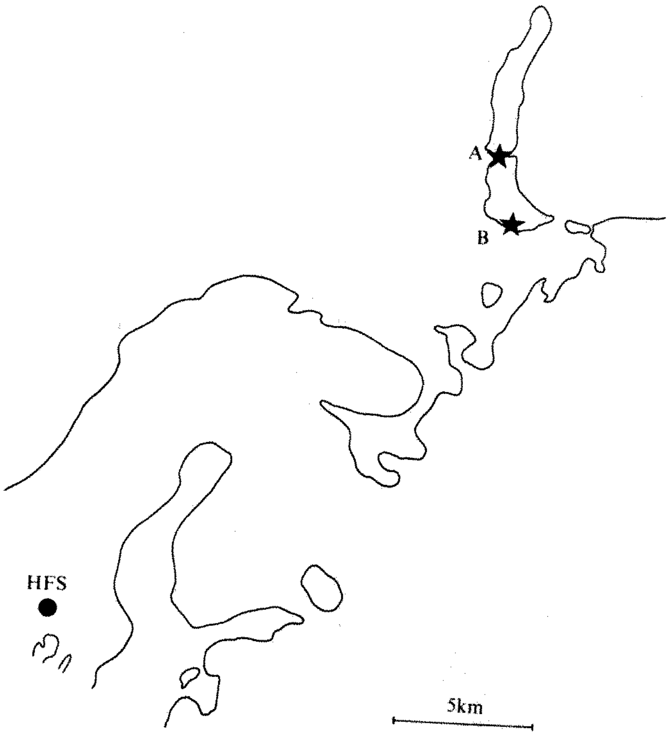

Fig. 1 Relative locations of the main (A) and the new (B) test sites on Novaya Zemlya and Hagfors Observatory (HFS).

Table 1 Location data of Novaya Zemlya events

	Year	Date Month	Day	Origin time h	min	s	Lat (N)	Long (E)	No. of stations in epicentre location	NOAA Lat (N)	NOAA Long (E)	m_b(HFS)	TMF (HFS)
Explosion	69	10	14	07	00	07	73.4±0.1	54.7±0.3	14	73.4	54.8	6.7	11.0
	73	09	12	06	59	54	73.1±0.1	55.6±0.3	13			7.8	6.0
	73	09	27	06	59	58	70.7±0.1	53.9±0.3	11	70.8	53.9	6.0	14.9
	73	10	27	06	59	57	70.8±0.1	54.1±0.3	12			7.3	8.1
Afterevent	73	10	27	07	52	25	71.1±0.2	54.0±0.6	6			4.0	10.5
				08	03	58	71.0±0.2	54.0±0.6	8			4.1	12.3
				08	09	35	70.7±0.3	54.8±0.9	6			3.8	13.6
				08	21	21	71.0±0.2	54.0±0.5	10			4.4	11.0
				08	23	59	70.5±1.2	55.2±2.0	5				†
				08	31	38*	70.8±0.5	55.4±2.0	4				†
				08	56	01*	71.4±0.3	53.2±0.8	7			3.9	9.7
				09	01	29	70.2±0.5	53.4±2.5	3				†
				09	13	49	71.1±0.2	53.8±0.4	11			4.4	10.1
				10	30	30*	71.8±0.5	57.8±1.9	5			3.7	13.2

*** Some stations have larger arrival time residuals than usual which means that the actual error might be larger than that given in the table.**
† Signals too weak to be analysed.

shocks detected by local seismic station networks from the deterioration of explosion cavities are very large. The Milrow and Cannikin explosions in the Aleutian Islands were followed by hundreds of events within a few hours of the explosions. These events were all small, most of them had seismic amplitudes less than 1/1,000 of those of the explosion[7]. These events are relatively weak compared to the events observed after high yield explosions at the Nevada Test Site, where the explosion Jorum for example generated fifteen events with amplitudes larger than 1/100 and forty events with amplitudes larger than 1/1,000 of the amplitude of the explosion[9]. The signals recorded by local networks from events related to the deterioration of the cavities were found to have a characteristic low frequency signature whereas tectonic events contain higher frequencies[7]. Significant spectral differences between teleseismic P waves from explosions and aftershocks associated with the explosion cavities at NTS have been reported[10].

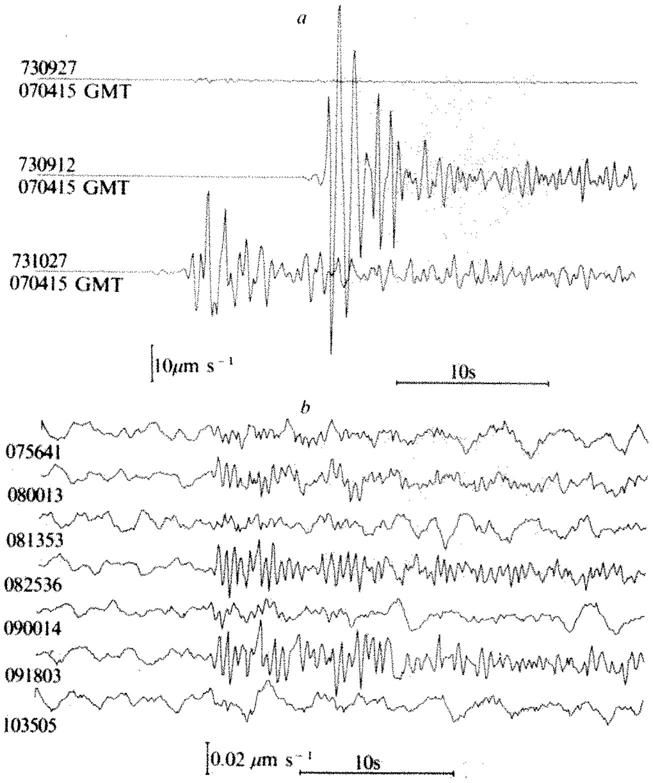

Fig. 2 *a*, Short period P signals recorded at the Hagfors Observatory in Sweden from Novaya Zemlya explosions; *b*, short period P signals recorded at the Hagfors Observatory in Sweden from aftershocks caused by the Novaya Zemlya explosion on October 27, 1973.

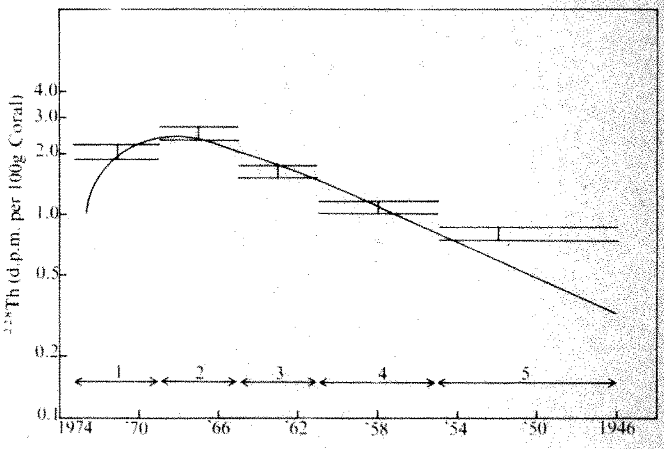

Fig. 3 Third moment of frequency, TMF, values as a function of body wave magnitude, m_b (HFS), for explosions (×) and aftershocks (○).

The aftershocks reported here from the Novaya Zemlya explosion on October 27 have the same general characteristics, maximum signal amplitudes of about 1/1,000 of that of the explosion and low frequency signals, as the aftershocks associated with the cavity deteriorations of the explosions in the Aleutian Islands. We thus conclude that the observed signals come from events related to the deterioration of the cavity of the explosion on October 27. No event which can be associated with the complete collapse of the cavity has so far been observed. The fact that no aftershocks have been observed from earlier large explosions at the old Novaya Zemlya test site might be explained by the difference in geology between the old and the new test site[11].

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Microfossils from the Late Precambrian Altyn Formation of Montana

SEDIMENTARY rocks of the late Precambrian Belt Supergroup are exposed in western Montana, Idaho, and adjacent parts of Canada. There are few reports of microfossils from the Belt Supergroup, although Metazoan ancestors may occur in the younger, upper Belt Missoula Group[1]. Early this century Walcott[2] concluded that stromatolites found in the Belt were produced by blue-green algae. He reported pyritised blue-green algal filaments, associated with stromatolites, from the Newland Limestone of lower Belt age. Recent re-examination of this material supported Walcott's interpretation[3]. I report here the discovery of microfossils, which are older than 1,300 m.y., from the lower Belt Altyn Formation.

The Altyn Formation is exposed only in the eastern part of Glacier National Park, Montana, and adjacent areas of Waterton Lakes Park, Canada. The formation is thrust over Cretaceous rocks along the Lewis Thrust, and the original sedimentary base is unknown. Elsewhere in Montana the Belt Supergroup rests unconformably on igneous and metamorphic basement rocks[4]. The Altyn Formation belongs to the lower Belt or Pre-Ravalli and is overlain by the progressively younger stratigraphic sequence: Ravalli Group, Helena Dolomite, Missoula Group. The formation is mainly dolomitic, with abundant terrigenous detritus at some horizons. It contains desiccation cracks, intraformational conglomerates, algal laminated sediments, oöids and algal stromatolites; indicating deposition in a shallow, nearshore marine environment. The Altyn Formation is a shoreline facies, deposited at the eastern margin of the Belt sea, and it grades westward into a finer-grained siliceous clastic facies[5]. Stromatolites, the product of a combination of physical and algal processes, have been known from the Altyn Formation for many years[6], but no fossil remains have been reported to date.

The Belt sediments were deposited during a 600 m.y. time span, between about 1,450 and 850 m.y. ago[4], with the Altyn Formation belonging to the lower, older part of the sequence. The Altyn Formation is not dated radiometrically, but some indication of its age may be obtained by considering radiometric ages of associated or correlative formations. The overlying Missoula Group is at least 1,100 m.y. old[7–9], and the Altyn Formation is some 2,700 m lower in the stratigraphic sequence[10], and must be older. The Aldrich Formation of British Columbia, a stratigraphic equivalent of the Altyn Formation, is intruded by the Hell-roaring Creek granite which is at least 1,300 m.y. old[4,11]. A second correlative of the Altyn Formation, the Prichard Formation in the Alberton, Montana, area, underwent regional metamorphism 1,330± 45 m.y. ago[4,9]. A suite of rocks from the Lower Belt of the Little Belt and Big Belt mountains has an age of 1,325± 15 m.y.[9]. Thus the Altyn Formation is at least 1,300 m.y. old, but is younger than the 1,700 m.y. old basement gneisses exposed beneath the Belt Supergroup in some places[4].

The microfossils occur in a siliceous grain (Fig. 1*a*), in a clastic dolarenite. The fossils were discovered in a thin section of rock, approximately 30 μm thick, prepared for petrographic, microscopic analysis. This implies that the fossils are an inherent part of the rock and not a later contaminant. Further evidence for this is the partial replacement of some filaments by early diagenetic dolomite (Fig. 1*a*, *b*), and the truncation of part of the thallus at the grain margin (Fig. 1*a*). The microfossils thus antedate the formation of the grain and the diagenesis of the sediments. The rock was sampled from a sequence of interbedded clastic dolarenites and algal laminated, finegrained dolomicrites, exposed near the top of Appekunny Falls, Glacier National Park, Montana.

The microfossils were preserved by the primary precipitation of amorphous silica. Subsequent recrystallisation to quartz provided a chemically and physically resistant medium for the organic material.[12] The microfossils demonstrate the effectiveness of this mode of preservation. The circular transverse sections show no evidence of distortion or compaction (Fig. 1*c*). The fossiliferous grain has withstood erosion and some intrabasinal transportation before its final deposition and lithification.

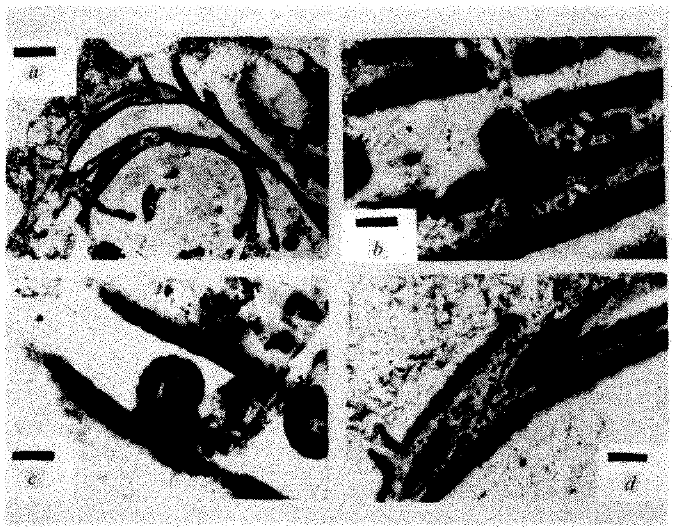

Fig. 1 Photomicrographs showing morphology of microfossils from the Altyn Formation. Bar scale: *a*, 100 μm; *b* and *c*, 10 μm; *d*, 20 μm.

The microfossils occur as a loosely coiled thallus composed of yellow-brown filaments. The filaments are tubular, non-septate, branched, occasionally straight, but more commonly curved (Fig. 1*a*), usually with rounded ends (Fig. 1*b*). Filaments are up to 1 mm long (incomplete filament) and circular in transverse section, with an average diameter of 16 μm (Fig. 1*c*). The filament walls have a quite uniform thickness averaging 6 microns. The filaments branch occasionally, with the angle between the two branches ranging from 15 to 90° (Fig. 1*d*). Intrafilament cellular material is not preserved, and mode of reproduction and the precise nature of the branching are unknown.

Comparison with previously described Precambrian microfossils reveals no forms which are directly comparable to the Altyn Formation microfossils. They are larger than *Archaeorestis schreiberensis*, a tubular, branched, non-septate filament of unknown affinities found in the Gunflint chert[13]. Fossils of the genus *Eomycetopsis* from the Bitter Springs Formation of Australia, and of unknown but possibly fungal affinities[12], are similar in some respects to the Altyn material. They are tubular, cylindrical, predominantly nonseptate filaments; however, they are unbranched and have filament diameters less than one fourth of those found in the Altyn Formation microfossils. Tubular, nonseptate fossils of the genus *Siphonophycus* from the Bitter Springs Formation are believed to be sheaths of a blue-green alga[12]. The Altyn Formation microfossils differ from those belonging to the *Siphonophycus* in their somewhat larger size and in being branched. In the absence of preserved intrafilament cells it is not possible to determine the type of branching. True branching is typical of eukaryotic plants, but did not develop in blue-green algae until the Devonian[14]. Definite assignment of the Altyn Formation microfossils is not yet possible, but it seems likely that they may be the preserved sheaths of blue-green algae with false branching.

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Calabrian Ridge, a newly discovered branch of the Mediterranean Ridge

LONG range side-scan sonar surveys made from RRS Discovery during 1971 and 1973 have provided the first regional view of the structural grain of the Mediterranean Ridge, whose distinctive surface relief was known previously only from echo-soundings[1–3]. The sonar surveys clearly show that there is an important grain extending along much of the Ridge, but also there is considerable local variation and other trends are well developed in places. Individual ridges and troughs of about 1–2 km wavelength, and crest length of at least 5 km, have been followed for up to about 25 km and, although largely parallel, they also branch and are noticeably sinuous. Near to the western end of the Mediterranean Ridge the structural grain turns round to the north (Fig. 1) and seems to terminate against a fault zone, which is close to the northern end of the Hellenic Arc[4] and to the northern limit of earthquakes in the Ionian Basin[5]. Extending south-westwards from this point a new branch, with a well developed grain in similar style topography, has been discovered. This dies out just before reaching the faulted, 3,000 m high Malta Escarpment. The inverted 'Y' shaped junction between these two Ridges encloses two sides of the triangular shaped Messina Abyssal Plain.

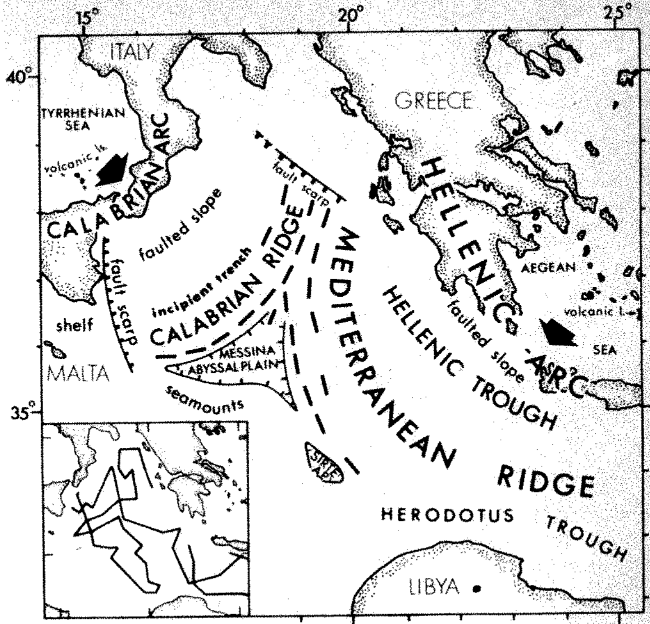

FIG. 1 Schematic diagram showing the inverted Y-shaped junction between the Calabrian and Mediterranean Ridges, together with the related structural elements bounding the Ionian Sea. The black bars provide representative indications of the grain of the ridges and troughs along the edges of the Messina abyssal plain, that were detected by long range side-scan sonar (Gloria). The inset figure gives the tracks along which Gloria sonographs were recorded, the line thickness about equalling the 14 km wide belt that was examined. The large arrows show the directions of inferred motion of the crust at the Hellenic and Calabrian Arcs with respect to the Ionian Sea.

This western branch we call the Calabrian Ridge, as it lies essentially concentric with, and is probably related in origin to, the Calabrian Arc (Fig. 1). The Calabrian Ridge is not a pronounced topographic feature, so its prior lack of recognition is not surprising. Its landward side is defined on bathymetric charts by a series of local deeps[6] marking the location of an incipient trench. An air gun profile extending from the western end of the Messina Abyssal Plain towards the toe of Italy shows a similar sequence across the Calabrian Ridge to that seen on crossings of the main Mediterranean Ridge. An acoustically transparent layer (Plio-Pleistocene, presumably) 600 m thick, assuming a two-way travel time of 2 km s^{-1}, overlies the 'M' reflector (Miocene evaporite sequence, presumably). At the abyssal plain both layers are only slightly disturbed, whereas on the Calabrian Ridge both the sea floor and the 'M' reflector are so broken that the latter appears to be diffuse. The narrow incipient trench (analogous to the series of deep trenches collectively known as the Hellenic Trough) has a flat floor underlain by up to 800 m of layered (and faulted) fill. Between this trench and the Italian coast alongslope tectonic trends are seen on sonographs, although without the distinctive texture of the Calabrian Ridge (as seen on sonographs and the air gun profile). This slope is not a depositional cone as previously thought[7], although there are some downslope trending canyons cut into it. The similarity of the Calabrian and Hellenic Arcs is further emphasised by the extremely narrow continental shelves and by the similar distance between the trenches and the inner volcanic arcs. The Hellenic Arc is currently associated with frequent shallow and intermediate depth earthquake activity, and the Calabrian Arc more with deep focus activity[4,8], which may suggest that the former is currently in a vigorous stage of activity and the latter in a dying phase. Thus, although the Calabrian Ridge need not necessarily further develop a pronounced relief, it nevertheless implies crustal shortening related to compression which is associated with the Benioff zone beneath the Calabrian Arc, probably as a result of south-easterly motion of the Tyrrhenian region. This is similar to the proposed relationship between the Mediterranean Ridge and the Hellenic Arc[5]. The inverted Y-shaped junction is thus the combined result of compression between the two arcs, which in travelling outwards are impinging on the Messina Abyssal Plain from two sides, accounting for the triangular shape of the Plain, since the southern side is bounded by a seamount chain.

We thank colleagues for operating and maintaining Gloria and air gun equipment at sea.

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Development of Playa Lakes in South Western Australia

SEDIMENT composition and provenance studies in ephemeral drainage systems of South Western Australia have shown interesting regional variation in the size, shape and orientation of playa lakes.

A major continental divide separates two drainage divisions in South Western Australia[1]. The South-west Division centres on the Avon and tributary systems, while the drier Eucla Division to the east is endoreic and contains many large playas. Here we describe the influence of winds on the morphology of playa lakes in the South-west Division, examples of which are shown in Fig. 1.

Figure 2 shows a size distribution plot for 600 playa lakes measured from aerial photo mosaics (1 : 63,360), twelve of which cover each topographic sheet (1 : 250,000) mentioned (Moora, Perenjori, and so on). The size range extends from features at the lower limit of measurement, 0.004 km^2 to several playa lakes over 100 km^2. The concentration around 0.05 km^2 reflects the presence of numerous small playa lakes in the South-west Division known locally as 'salt pans'. A characteristic of these playa lakes is the recurrence of certain shapes, commonly elliptical, obloid, circular and elongate. Figure 3 shows how the shape index E ($E = (L-W)/L$, where E=ellipticity, L=long axis length and W=short axis length[2]) varies within the same drainage systems. There is a significant concentration of values around $E=0.33$.

Regional contrast is evident in preferred long axis orientation measured against 'photo' north (Fig. 4). In the north and closer to the west coast (Moora/Perenjori) many playa lake features are orientated slightly west of N (at a mean orientation of 347°). Inland long axis orientation is east of N and in the southern drainage systems (Corrigin, Hyden, Newdegate) mean long axis orientation is 33° east of north.

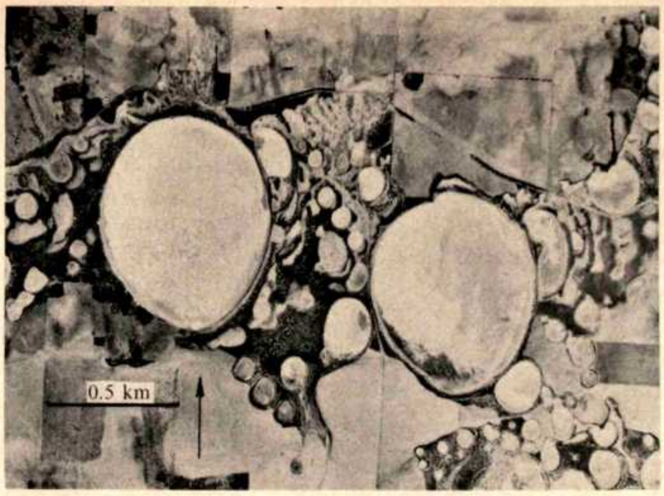

Fig. 1 Playa lakes near Moora, South Western Australia.

Hydrodynamic analysis of shaping processes in shallow lakes in unconsolidated sediment is well documented[3–7]. Such studies show that consistency of orientation, high ellipticity values and smooth curvature of shoreline are equilibrium forms of wave generated currents, reflecting zones of maximum mean current velocities around margins. Bruun[3] indicated that maximum littoral drift occurs on downwind shores where angles between wave orthogonals and normals to shoreline are 50°. Minimum littoral drift occurs where these angles are 0°. For shorelines of limited length a cycloid equilibrium outline results which is orientated normal to dominant winds. Reversal of dominant winds produces two opposite shorelines of cycloid form forming a basin of elliptical shape[4]. In an effort to explain the data in Figs 2–4 we tested the applicability of this model derived from analysis of current flow on beaches and in permanent lakes in relation to playa features aperiodically flooded. Following the flooding of many local South-west Division playas during winter 1973, rhodamine B dye was used to

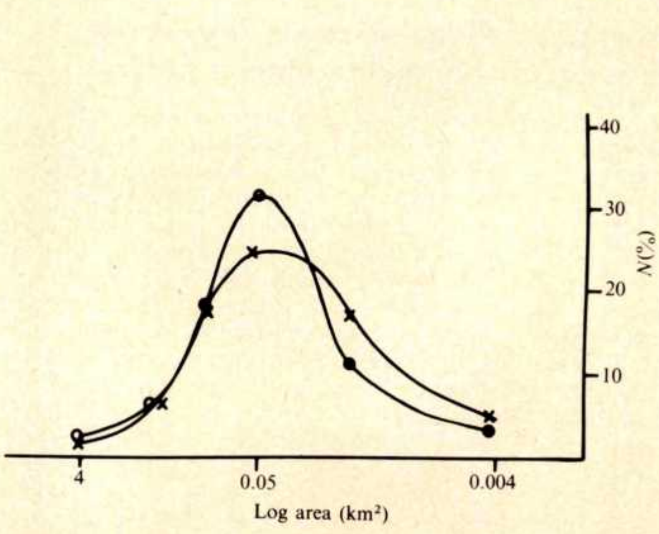

Fig. 2 Size distribution of six hundred playa lakes in South Western Australia measured from 1 mile to the inch air photograph mosaics. Place names refer to 1 : 250,000 topographic maps covered in this study. ○, Hyden, Corrigen, Newdegate; ×, Moora, Perenjori.

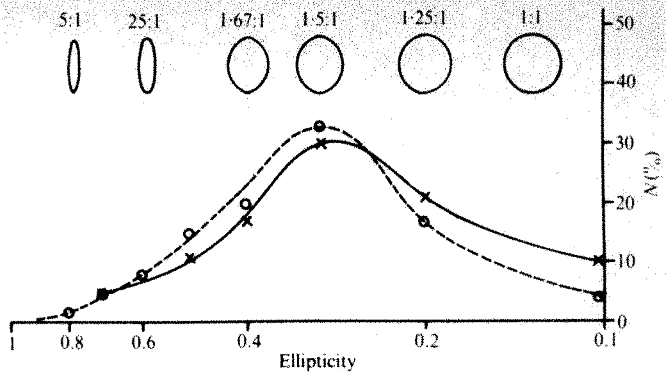

Fig. 3 Shape distribution of 600 playa lakes in South Western Australia measured from 1 mile to the inch air photograph mosaics. Place names refer to 1 : 250,000 topographic maps covered in this study. ○, ×, As Fig. 2.

track and measure currents in about 10 cm of water during winds of velocities up to 900 cm s^{-1}. Fine colloidal streamers of sediment are agitated into suspension and moved by littoral currents. Patterns and velocities of flow agree in general with current measurements of Carson and Hussey[4]. Sorting analysis of sand from small sandy beach deposits around many playas also reflects velocity differentials.

In South Western Australia wind velocities and magnitudes have been measured since 1964, as two daily Beaufort Scale recordings over sixteen compass points. From this data averages for both twelve month and six winter months (May–October) periods were made for twenty centres in the South-west Division.

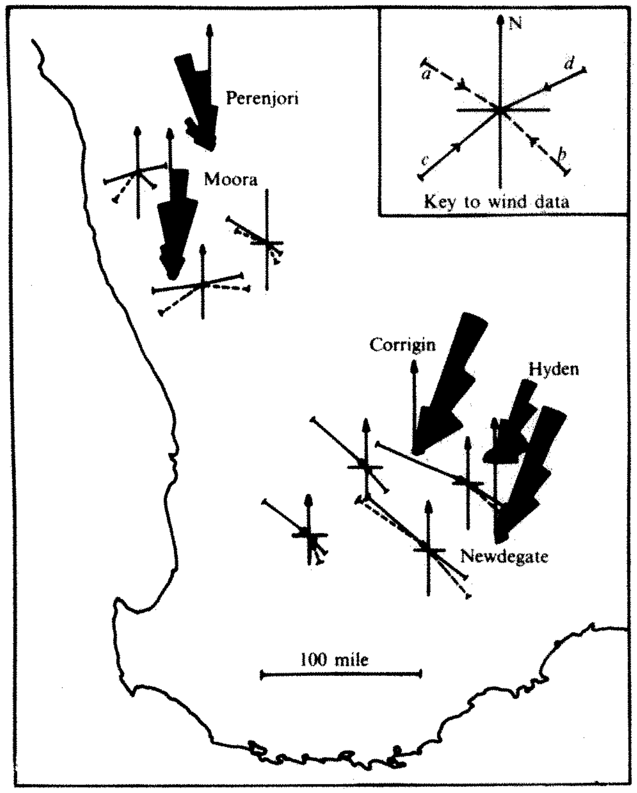

Fig. 4 Rosette diagrams showing long axis orientation of playa lakes in South Western Australia. Wind vector resultants about mean playa lake orientation are also shown for adjacent centres (*a*, Western component 12 month data; *b*, eastern component 12 month data; *c*, western component for 6 winter months; *d*, eastern component 6 winter months. Where 12 month and 6 winter month vectors are within 5° of each other only the winter vector is shown.)

It was argued that if local playa lakes had a sufficiently high flooding ratio[8]—the number of days they contain water each year/365—and if as indicated, the Bruun model is generally applicable, a correlation should exist between winter-wind resultants and playa lake orientation. Final resultants ought to parallel the mean short axis orientation in a particular region, and should be approximately opposed. Such correlations have been shown to exist in shallow lake systems elsewhere[4,5]. In the areas investigated a 46° variation in regional long axis orientation is indicated and hence simple vector resolution about N–S as used by Carson and Hussey[4] is pointless if the object is to test the above relationships. But when vector resolution is made about mean playa long axis orientation for a particular area, winter wind resultants remain approximately normal to long axis orientation and opposite each other. Resolutions about other directions do not give this result.

Figure 4 shows the regional long axis orientaion of playa lakes in the South-west Drainage Division. Also shown are wind vector resolutions for selected sites made around the mean long axis orientation of nearby playa lakes. As indicated a sharp contrast is evident in both sets of data between the northern and southern parts of the division. Within the Moora/Perenjori set long axis orientation has a north-north-western alignment in the coastal examples, while inland long axis orientation swings east of north. In the southern part of the division (Corrigin, Hyden, Newdegate) inclination of long axis to the north-north-east is well developed, though an increasing variability is evident with increasing aridity (such as Hyden).

Wind vector resolution around the mean long axis orientation of local playa lakes consistently produces near normal resultants for winter winds. A greater variation in the corresponding twelve months data is observed. This close agreement of winter wind vector resultant and normals to long axis orientation occurs in both the north coastal and inland examples.

Studies of fluvial systems have encouraged the view that process and sedimentary medium interact to produce a continuum of drainage patterns[9]. Drainage features in ephemeral systems are an important set in such a continuum, comprising a variety of related forms.

The evidence described above suggests that playa lake features within the ephemeral drainages systems of the South-west Drainage Division display an equilibrium adjustment of shape, size and orientation to contemporary fluvioaeolian processes. In spite of aperiodicity of rain, flooding ratios are apparently high enough to shape and orientate playa lakes. The relative position and form of margin deposits, both beach and lunette, appear to be related to Bruun's[3] maximum/minimum zones of current erosion and deposition, with subsequent movement by wind.

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Support for atmospheric origin of anomalous submillimetre wave spectral features

A RECENT letter in *Nature*[1] showed a feature at a frequency of 8 cm^{-1} in one of two spectra of the atmosphere. That the feature is an artefact follows from the authors' own statement that no true spectral observation was possible. The indication was found only in records from daylight hours and the proposal was made that it was caused by some solar process with an appropriate time variation coupling to the recording system in some unspecified way. We do not wish to comment on this process or on the authors' own evidence given to support it. But they also quote published results as giving further support to their hypothesis with the clear implication that features in the spectra of other workers may be due to the same kind of artefact. We contest this since it is easy to distinguish between a true spectral indication and an artefact, coming from temporal variations of source or absorption intensity, by varying the time rate of change of path difference given by the interferometer.

TABLE 1 Properties of spectra in Fig. 1

	Curve *A*	Curve *B*	Curve *C*
Precipitable water (mm)	0.85	0.85	1.84
Path length (m)	80	80	20.25
Temperature (K)	293	293	328
Resolution (cm^{-1})	0.25	0.25	0.5
Transmission value at 8 cm^{-1}	0.95	0.75	0.60

Baluteau *et al.* cite evidence (see ref 2) with the mistaken supposition that the results came from measurements using the Sun. It was clearly stated that spectra were obtained using an artifical source and a horizontal atmospheric path and, to add to the record, the spectra were recorded in the hours of darkness. The argument for this work supporting their hypothesis is therefore invalid.

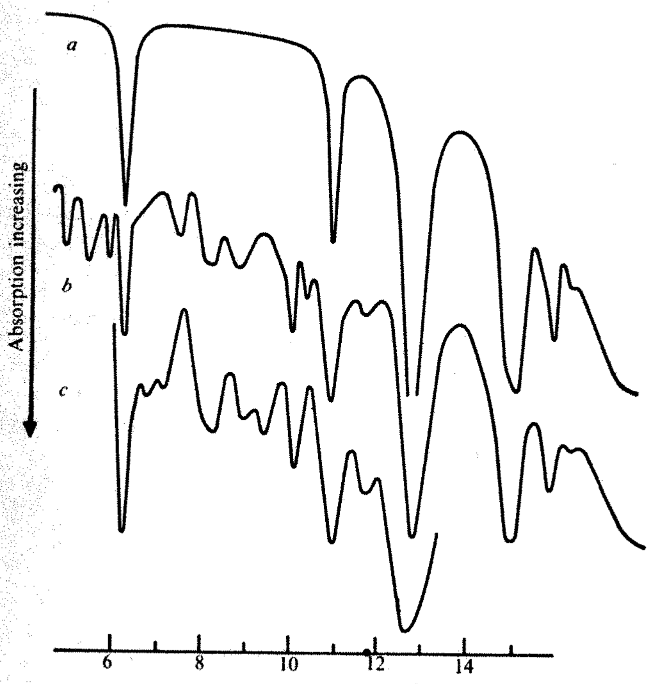

FIG. 1 Water vapour absorption spectra. *a*, Theoretical spectrum for monomeric water. *b*, Measured spectrum for a horizontal path in the atmosphere. *c*, Laboratory spectrum for water vapour alone. See Table 1.

In stating that "effectively laboratory spectra" do not show any anomalous peak in this spectral region Baluteau *et al.* seem to have overlooked other work of ours (ref. 3). Though this gave somewhat lightweight evidence it seems to have been essentially correct and provided an important clue about anomalous absorption. New work which relates to it is shown in Fig. 1 curve *c*.

The topic of anomalous millimetre and submillimetre absorption is being closely studied at the Appleton Laboratory. The two observed spectra (Fig. 1 curves *b* and *c*; Table 1) which are taken from work which will later be published in full, support our view that anomalous absorption is an atmospheric phenomenon and that the molecular complexity of water vapour is an important, if not the only factor in explaining it.

This work is part of the programme of the Appleton Laboratory.

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Safety in the preparation of multimetallic catalysts

THERE is presently an extensive research effort in the field of catalysis concerned with alloy systems. Many researchers are preparing and characterising unsupported systems in order to study the metal phase in the absence of the support. A classical approach to doing this is that of simultaneous chemical reduction of the salts of a given alloy system in aqueous solution. Such reducing agents as hydrazine or sodium borohydride are frequently used. During the course of our work we found that the reduction of ruthenium salts with borohydride solutions gave a 'metal powder' which, when dried, violently exploded on touching it with water and sometimes by simple stimulation with a spatula. Under certain conditions a very unstable and poorly characterised 'ruthenium hydride' is apparently formed. A safer alternative technique is to use aqueous solutions of hydrazine for the chemical reduction of ruthenium-containing solutions.

Along these same lines, supported metal catalysts which contain gold should never be prepared by impregnation of a support with solutions which contain both gold salts and NH_4OH. The dried catalysts contain extremely shock-sensitive gold-nitrogen compounds which may explode with the lightest touch.

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BIOLOGICAL SCIENCES

Subcellular Separation and Molecular Nature of Human Histocompatibility Antigens (HL-A)

HISTOCOMPATIBILITY antigens have been solubilised by various means[1], especially proteolytic digestion[2–4], sonication[5] and extraction with 3 M KCl[6] or detergents[7–8]. Although detergents seem to solubilise the intact antigens these preparations have, until recently, proved difficult to fractionate further, whereas the products of the other procedures have proved amenable to purification but probably represent fragments of the membrane-bound antigen. This interpretation is based on the following arguments. First, solubilisation with 3 M KCl seems to depend on the presence of cell sap and was markedly reduced by protease inhibitors[9]. Second, in contrast to the detergent-solubilised antigen[10], the other procedures yield non-aggregated products which, in general, possess similar molecular weights within the range 35,000–55,000[10–12]. Third, mouse and human histocompatibility antigens obtained by papain digestion seem to be glycoproteins (3–8% carbohydrate), whereas the antigens solubilised from guinea pig and human tissues by sonication and 3 M KCl extraction have been reported to be non-glycosylated (less than 1% carbohydrate)[12]. This dichotomy would be explicable if the latter procedures had caused degradation and the consequent separation of the carbohydrate from the molecule bearing the antigenic determinant.

If the above views are correct then conclusions on the molecular nature of histocompatibility antigens adduced from these non-detergent solubilised preparations are questionable. Solubilisation procedures relying on degradation also have other disadvantages. The yield of antigen is usually low, fragments are generally less immunogenic than the whole molecule and may induce a different spectrum of immune responses (for example, cellular as against humoral), and the products are not suitable for determining the antigen's mode of integration into the cell surface membrane. These disadvantages have led us to develop an alternative approach using a highly purified preparation of the surface membrane of a human lymphoblastoid cell line as the source of HL-A antigens, and solubilising the antigens completely with sodium deoxycholate. This detergent circumvents the difficulties associated with the strongly ionic detergents such as sodium dodecyl sulphate (SDS). It has also enabled separation of the HL-A antigens from the majority (90%) of the membrane proteins and the estimation of some of the molecular parameters of the apparently intact antigens. Similar approaches based upon the use of the non-ionic detergent, NP-40, have recently been reported[8,10]. A comparison of the results reveals some interesting differences in the apparent molecular sizes of the antigens solubilised by deoxycholate and NP-40.

Antigenic activity was assessed by inhibition of fluorochromatic cytotoxicity[13] using microtest plates (Searle Diagnostics, High Wycombe, Bucks.). The choice of this assay was based on its applicability to whole cells, subcellular fractions, and solubilised materials (Table 1), and its reproducibility (±20% for three separate estimations for deoxycholate-solubilised membrane over one month). Antigen (1 μl) was incubated under mineral oil for 1 h at room temperature with 1 μl of antiserum before addition of fluorescein-labelled, typed, peripheral blood human lymphocytes as target cells. Quantitation was achieved by titrating several dilutions of the antigen against nine dilutions of antiserum; the endpoint for each antigen dilution was the antiserum dilution giving 50% killing of the target cells compared with the controls without antiserum. These endpoints were plotted according to Reif's modification of von Krogh's equation[14]; linear plots were obtained and the intercept on the y axis represented the amount of antigen giving 50% inhibition (ID_{50}).

Antigens of the LA series were assayed using sera 'Yochum' for (HL-A2); 'Dovey' and 'Gillespie' for HL-A1; while sera 'Dovey' 'P1422B', and 'P1305B' were used for HL-A8 and 'P1040B' for HL-A13 of the series 4 antigens. The antisera were rendered effectively monospecific by using appropriate target cells. The possibility that these sera detected antigens unique to BRI 8 cells was excluded by using a panel of typed peripheral blood lymphocytes to define the antisera specificities, and as target cells in the assay. It has been suggested previously that cytotoxicity is facilitated by a heterophile antibody present in the rabbit complement[15]; in this case, if BRI 8 cells absorbed this antibody or were anti-complementary by another mechanism, then nonspecific inhibition would occur. This was ruled out by demonstrating that previous adsorption of the complement (10^7 BRI 8 cells per ml equivalent to 50 ID_{50} units in the assay, for 1 h at 4° C) had no discernible effect on the antigen titre. The problem of nonspecific killing of the target cells by the deoxycholate added with solubilised antigens was circumvented either by diluting the fractions until they were no longer cytolytic, by diluting the antiserum in a gross excess of extraneous protein (20% foetal calf serum) or by previous removal of the detergent using Amberlite XAD-2; in general, a combination of the first two procedures was used.

Although H-2 and HL-A antigens have been claimed to be associated with intracellular membranes, various results suggest that they are located mainly on the cell surface. As a result, a purified preparation of the surface membrane was chosen as the antigen source since, in this way, degradation by cell sap[9] before or during solubilisation should be minimal.

BRI 8 cells, a diploid lymphoblastoid cell line derived from

Table 1 Recovery of HL-A2 antigenic activity on cell disruption, extraction of cells with 3 M KCl and solubilisation of the plasma membrane with 1% Na deoxycholate

Material	Initial HL-A2 activity* (ID_{50} total units)	Treatment	Recovered HL-A2 activity* (ID_{50} total units)	Recovery (%)
BRI 8 cells 8×10^9	7.3×10^6	Disruption†	7.3×10^6	100
BRI 8 cells 7.6×10^{10}	7.0×10^7	Disruption†	8.3×10	118
BRI 8 cells 2×10^9	1.8×10^6	Extraction 3 M KCl‡	2.4×10^5	12
BRI 8 plasma membrane	6.7×10^5	Solubilisation by Na deoxycholate§ Soluble	6.0×10^5	90
		Insoluble	3.4×10^3	1

* Assayed using serum 'Yochum'.
† As described for the preparation of plasma membrane (see text); these figures are for two different preparations.
‡ As described by Reisfeld *et al.*[6].
§ Plasma membrane was incubated for 30 min at room temperature in 1% Na deoxycholate and then centrifuged at 100,000*g* for 1 h as described previously[29].

Table 2 Distribution of protein, HL-A2 antigenic activity and 5′-nucleotidase on fractionation of disrupted BRI 8 cells

Fraction	Protein (mg total)	5′-Nucleotidase activity Total (μmol Pi/h)	5′-Nucleotidase activity Specific (μmol Pi/mg protein/h)	HL-A2-activity* Total (ID_{50})	HL-A2-activity* Specific (ID_{50}/mg protein)	Ratio 5′-nucleotidase/ HL-A2 ($\times 10^4$)
Homogenate (4×10^{10} cells)	5,520	552	0.10	3.5×10^7	6.3×10^3	6.3
Pooled nuclear and mitochondrial	3,410	170	0.05	1.4×10^7	4.2×10^3	8.4
Microsomal			1.93		1.0×10^5	5.2
Supernatant	2,190	44	0.02	3.5×10^6	1.6×10^3	8.0
Endoplasmic reticulum †	54	49	0.91	—	—	—
Plasma membrane †	34	174	5.10	5.1×10^6	1.5×10^5	3.0
Unretarded ‡	6.3	0	0	0	0	—
Glycoprotein ‡	0.64	20.5	32.0	1.4×10^6	2.2×10^6	6.8

Fractionations were carried out as previously described (ref. 20 and M. J. Crumpton and D. Snary, in preparation) using differential centrifugation, sucrose density gradients and LcH columns.

* Assayed using serum 'Yochum' but similar results were obtained with serum 'Dovey'.

† Separated from the microsomal fraction by sucrose density gradient centrifugation (Fig. 1).

‡ Separated from deoxycholate-solubilised membrane (7.5 mg membrane protein) by chromatography on LcH-Sepharose (Fig. 2).

normal human peripheral blood [HL-A antigens: 1,2,8,13 (4a, 4b)], were purchased from Searle Diagnostics. Cells were grown in spinner culture in RPMI 1640 medium supplemented with 10% (v/v) foetal calf serum and were collected while growing logarithmically since membrane prepared from terminal cultures showed by polyacrylamide gel/SDS electrophoresis evidence of degradation. The cells collected were resuspended in Eagle's minimal essential medium (MEM) (5×10^7 to 2×10^8 cells per ml of MEM) and were disrupted at 0° C by extrusion at a rate of about 400 ml h^{-1} through a small orifice under a pressure of about 750 pound inch^{-2} against a spring-loaded ball (developed from a design by Dr P. M. Wright). Under these conditions the cell number was reduced by about 50% and the viability, as measured by trypan blue exclusion, decreased from about 85% to 5%; electron microscopy revealed whole nuclei and cells denuded of their surface membrane. Table 1 shows that disruption was not associated with any significant change in the amount of HL-A2 antigen. This result agrees with that reported previously for H-2 antigens[16,17] and is consistent with the view that histocompatibility antigens are expressed mainly, if not exclusively, at the cell surface.

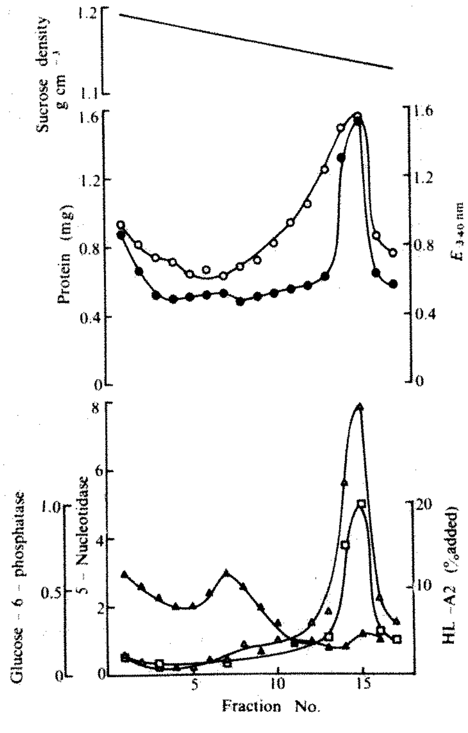

Fig. 1 Fractionation of microsomal pellet by sucrose density gradient centrifugation. The microsomal fraction was resuspended in 3.5 ml of 10 mM-Tris-HCl buffer, pH 7.4, by using a tight-fitting homogeniser and 1 ml samples were layered onto continuous sucrose gradients [8 ml of 25% (w/v) against 8 ml of 42%]. After centrifuging at 22,000 r.p.m. for 18 h in a SW27 rotor, 1 ml fractions were removed from just above the small pellet by using a Perpex pump. Samples were analysed for sucrose density, turbidity (●, E_{340nm}), protein (○), 5′-nucleotidase (Δ, μmol Pi per mg protein per h), glucose 6-phosphatase (▲, μmol Pi per mg protein per h) and HL-A2 activity (□).

The homogenate was fractionated as previously described[18] by differential centrifugation (Table 2) and the microsomal fraction was subsequently purified by centrifuging on a continuous or discontinuous sucrose density gradient (Fig. 1). All operations were performed at 0° C and as quickly as possible to reduce degradation to a minimum. Addition of diisopropylfluorophosphonate in isopropanol to the microsomal fraction (1 μl of a molar solution per ml) was discontinued when it was noticed that it caused aggregation of membrane vesicles. The most notable feature of the results shown in Table 2 is the very close similarity in distribution of HL-A2 antigen and the plasma membrane marker, 5′-nucleotidase, amongst the various subcellular fractions. This close association is confirmed in Fig. 1 which shows that these activities were distributed in an identical manner on sucrose density gradient centrifugation of the microsomal fraction. Furthermore, both activities were concentrated in the leading edge of the protein peak with a sucrose density of 1.14 g cm^{-3} which is characteristic of plasma membrane[18] and neither activity was present to any significant extent in the band of higher sucrose density that possesses the endoplasmic reticulum marker, glucose 6-phosphatase. These results confirm and extend those reported recently by Wilson and Amos[19] and emphasise the more or less exclusive association of HL-A antigens with the plasma membrane.

The plasma membrane fraction consisted predominantly of smooth membrane vesicles which according to various morphological and biochemical criteria were not contaminated by significant amounts of nuclei, mitochondria, endoplasmic reticulum, lysosomes, ribosomes or cytosol (M. J. Crumpton and D. Snary, in preparation). The purified membrane was suspended in 10 mM Tris-HCl buffer, pH 7.4, or dissolved in 1% Na deoxycholate in 10 mM Tris-HCl buffer, pH 8.2 and stored at −70° C. Alternatively, small aliquots (10–40 μl) of membrane suspension, solubilised membrane or 3 M KCl extracts[6] were stored in plastic straws plugged with plasticine in liquid N_2 (ref. 20). Storage of the membrane suspension at 0° C or successive freezing and thawing gave marked reductions in HL-A activity and in solubility in deoxycholate. These changes were probably due to degradation since on polyacrylamide gel/SDS electrophoresis the aged membrane gave more intensely staining low molecular weight bands and fewer high molecular weight bands than the fresh membrane. In this case, in view of the membranes' high degree of purity it seems likely that this degradation is an intrinsic property of the membrane. In contrast, the HL-A activity of solutions of the membrane glycoproteins (see below) in deoxycholate was stable at −20° C and to successive freeze-thawing.

Recently a method was described for the isolation of membrane glycoproteins by means of affinity chromatography of deoxycholate-solubilised membrane on *Lens culinaris* phytohaemagglutinin (LcH) covalently attached to Sepharose[21,22]. LcH possesses an antibody-like specificity for glucose, mannose and sterically-related sugar residues[23]. If, as seems likely, HL-A antigens are glycoproteins then this method should be applicable to their separation from the membrane lipids and non-glycosylated proteins.

The purified plasma membrane was solubilised in 1% Na deoxycholate (4 mg of membrane protein ml^{-1}) by incubating for 30 min at room temperature followed by centrifuging at 100,000*g* for 1 h. The supernatant fraction contained about 90% of the membrane protein, 5′-nucleotidase and HL-A2 antigenic activity. In view of the almost complete recovery of HL-A2 activity on solubilisation (Table 1) it seems, in contrast to previous work using NP-40[8], that serum 'Yochum' recognises membrane-bound and solubilised antigen equally well. The fractionation of the deoxycholate-solubilised membrane using LcH-Sepharose is illustrated in Fig. 2. About 10% of the added protein was bound to the column and was subsequently eluted with methyl-α-D-mannopyranoside. The eluted fraction possessed the majority (87%) of the added HL-A2 activity (Table 2) and all of the known membrane glycoprotein marker, 5′-nucleotidase[24]. In contrast, the unretarded material (fraction No. 4, Fig. 2) contained no detectable 5′-nucleotidase activity and also failed to inhibit cytotoxicity due to serum 'Yochum' at a dilution of 1 : 5 or at 1 : 1 after removal of deoxycholate using Amberlite XAD-2. In view of the distribution of 5′-nucleotidase activity and the known specificity of LcH[23], it was concluded that all the HL-A2 antigen was glycosylated. This conclusion is contrary to that derived from the analysis of HL-A antigens solubilised by sonication or 3 M KCl extraction[12] and is consistent with the suggestion that the latter preparations had undergone partial degradation.

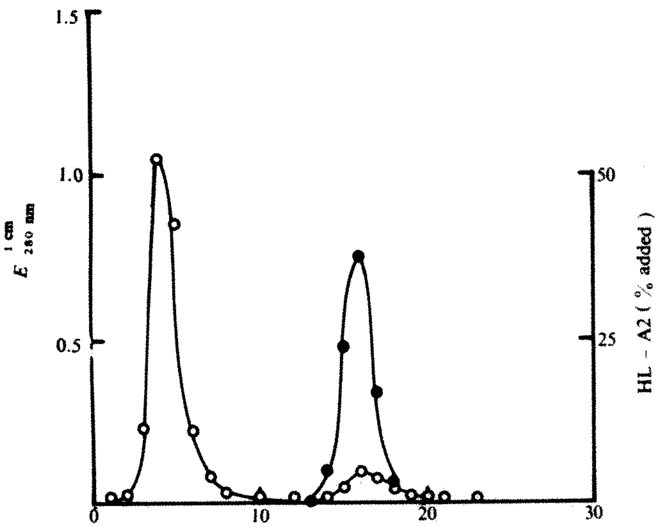

Fig. 2 Fractionation of BRI 8 plasma membrane using LcH-Sepharose. Membrane containing 8 mg of protein was incubated for 30 min at room temperature with 2 ml of 1% Na deoxycholate in 10 mM Tris-HCl buffer, *p*H 8.2, and centrifuged at 100,000*g* for 1 h. The supernatant (7.5 mg of protein) containing about 90% of the HL-A and 5′-nucleotide activities was eluted from a 10 ml column of LcH-Sepharose with 1% deoxycholate until the extinction of the eluate at 280 nm returned to the base line. The column was then washed (fraction No. 12) with 2% (w/v) methyl-α-D-mannopyranoside in 1% deoxycholate. Fractions (2.7 ml) were assayed for protein (○) and for HL-A2 activity (●). HL-A activities corresponding to less than 0.8% of the activity added to the column would not have been detected under the assay conditions used.

The yield of HL-A2 activity in the glycoprotein fraction relative to that of the cell homogenate varied from 15 to 40% and the overall purification was about 350-fold; the variation in yield depended solely upon the recovery of plasma membrane which was apparently inversely related to the number of cells used, in that the higher yields of membrane were invariably obtained using smaller numbers (8×10^9) of cells. If it is assumed that approximately 1.0% of the membrane protein is HL-A antigen (about 4×10^5 mol per BRI 8 cell) then 10% of the glycoprotein fraction is HL-A. Comparisons of the above yield with those achieved previously for the same degree of purification are difficult due to lack of information. The present yield of membrane-bound antigen would, however, seem to be comparable with the highest recovery (about 16%) reported for the papain-solubilised antigen[5] and, in some cases, to be noticeably superior (a yield of 1–2% for 350-fold purified papain-solubilised H-2 antigens[3]). Although higher recoveries have been claimed for 3 M KCl-extracted antigens[6], this technique gave yields of 12% only (Table 1) in the present study.

Preliminary attempts to exploit lectins with different carbohydrate specificities to sub-fractionate the glycoproteins further have met with limited success. For example, *Axinella polyploides* agglutinin (M. J. Crumpton and A. P. MacLennan, in preparation), which is specific for D-galactose residues, absorbed 4% of the membrane protein (that is, about 40% of the glycoprotein fraction) but none of the HL-A2 activity.

The molecular sizes of the membrane-bound HL-A antigens were estimated by sucrose density gradient centrifugation[25] of deoxycholate-solubilised BRI 8 plasma membrane in the presence of 1% sodium deoxycholate. The HL-A2 activity was distributed in an essentially symmetrical peak; the small pellet contained no detectable HL-A activity (Fig. 3). A comparison of the width of the peak with that of the membrane 5′-nucleotidase (Fig. 3) or a marker protein, aldolase, suggested however, that the HL-A2 antigen was slightly polydisperse. $S_{20,w}$ values were calculated relative to those of typical, water-soluble, globular proteins (such as, aldolase, bovine serum albumin), and are based on the assumption that the HL-A antigens and marker proteins possess similar partial specific volumes and molecular shapes in deoxycholate; this assumption is only valid if both groups of molecules do not bind the detergent or if they bind similar amounts (see below). The results for the reduced and unreduced, membrane-bound series LA and 4 antigens are summarised in Table 3; the values for the papain-solubilised HL-A antigens in deoxycholate are included for comparison.

The above results are notable for a number of reasons. First, the series LA and 4 activities sedimented at slightly different rates as judged using various anti-LA and 4 sera. This difference could be due to for example, series 4 antigens not being more readily degraded than LA antigens, or LA and 4 antigens having similar molecular sizes but binding different amounts of deoxycholate (that is, LA antigens may be integrated more deeply into the membrane lipid bilayer). Perhaps the simplest explanation, however, is that the LA antigens have a larger molecular size than the 4 antigens. This explanation is consistent with the report that in the mouse the antigenic specificities governed by the H-2K and H-2D genetic regions are expressed on independent molecules with slightly different molecular weights (44,000 and 39,000 respectively)[10].

Second, the $S_{20,w}$ value for papain-solubilised HL-A antigens in deoxycholate (3.68S) agrees closely with that determined for papain-solubilised H2 antigens in the absence of detergent (3.6S)[11]. This agrees with the report that water-soluble proteins (the marker proteins and papain fragments) bind little or no deoxycholate[26].

Table 3 Comparison of $S_{20,w}$ of membrane-bound and papain-solubilised HL-A antigens of the LA and 4 series in 1% Na Deoxycholate

Material	$S_{20,w}$* of HL-A antigens LA: series †	4 series ‡
Deoxycholate-solubilised BRI 8 plasma membrane	5.15	4.55
Papain-solubilised§	3.68	3.68
Mercaptoethanol treated deoxycholate-solubilised BRI 8 plasma membrane‖	5.24	4.35

* Estimated by sucrose density gradient centrifugation, as described by Martin and Ames[25], relative to the sedimentation of aldolase and bovine serum albumin under identical conditions; experimental details are given in the legend of Fig. 3. The figures in the table were obtained from separate tubes in a single centrifuge. Determinations of the $S_{20,w}$ for the LA series in 4 separate experiments were within range of 0.05 and two determinations for the 4 series agreed, to within experimental error (<0.05).

† Estimated using sera 'Yochum' and 'Dovey'.

‡ Estimated using 'Dovey', 'P1422B', 'P1305B' and 'P1040 B'.

§ A mixture of HL-A2 and HL-A7 obtained from separate spleens by papain digestion in the presence of 5 mM cysteine[2] and donated by Dr A. R. Sanderson.

‖ Membrane solubilized and centrifuged under reducing conditions in the presence of 2-mercaptoethanol.

Third, the deoxycholate solubilised HL-A antigens sedimented very much faster than the papain-solubilised material. This difference was not due to the reducing conditions (5 mM cysteine) used during papain digestion since treatment of the solubilised membranes with 2-mercaptoethanol had no discernible affect on the antigens' rates of sedimentation. Alternatively, it seems reasonable to assume that the difference is related to the presence in the membrane-bound antigens of a hydrophobic region which is necessary for insertion of the antigens into the membrane and which is detached during solubilisation by proteolysis (compare the influenza virus membrane glycoproteins[27,28]). The actual difference in molecular size between the membrane-bound and papain-solubilised antigens is impossible to assess without knowing whether the whole antigen binds deoxycholate and, if so, how much. The results of Helenius and Simons[26] using erythrocyte membrane proteins as a model predict that the HL-A antigens should bind about 0.38 g of deoxycholate per g protein. In this case their rates of sedimentation would correspond to molecular weights of about 54,500 and 45,200 for the LA and 4 series respectively. On the other hand, it is questionable whether the HL-A antigens actually bind significant amounts of deoxycholate; thus, they failed to form the very large aggregates detected by Helenius and Simons[26]. Also, not all membrane proteins apparently bind deoxycholate; for instance, the membrane 5′-nucleotidase behaves in deoxycholate (Fig. 3) as if it had a molecular weight of about 160,000 which corresponds closely to that derived for the mouse liver enzymes[24]. In this case the molecular sizes of the LA and 4 antigens (88,000 and 73,000 respectively) are in close agreement with that reported for the 'dimeric' H-2 antigen (molecular weight 88,000) detected by polyacrylamide/SDS electrophoresis of NP-40 solubilised material[10].

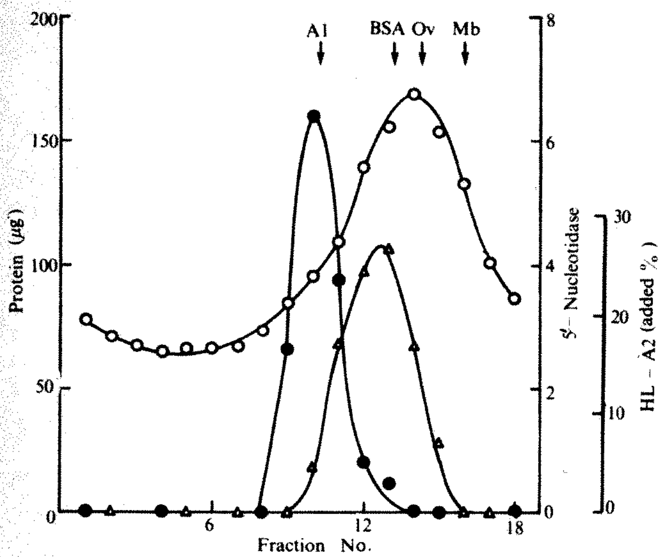

Fig. 3 Sucrose density gradient centrifugation of membrane-bound HL-A2 antigen dissolved in 1% Na deoxycholate. BRI 8 plasma membrane (28 mg of protein) was incubated for 30 min at room temperature with 7 ml of 1% Na deoxycholate in 10 mM Tris-HCl buffer, *p*H 8.2 and 1 ml of the mixture was layered onto a linear sucrose gradient [5 ml of 9% (w/v) against 5 ml of 28%] containing 1% Na deoxycholate. Marker proteins [aldolase (A1) 7.5S; bovine serum albumin (BSA) 4.41S; ovalbumin (Ov) 3.55S; sperm whale myoglobin (Mb) 2.08S] were added to identical gradients. The gradients were centrifuged at 4° C in a Spinco SW 41 rotor for 48 h at 38,000 r.p.m. (180 g_{av}). Timed fractions (0.67 ml) were removed from the bottom of the tube using a Perpex pump. Samples were assayed for protein (○), 5′-nucleotidase (●, μmol Pi per mg protein per h) and HL-A2 activity (△) using serum 'Yochum'; serum 'Dovey' gave an identical distribution of HL-A1 activity. The small pellet at the bottom of the tube contained no detectable HL-A2 activity (see also Table 1).

Fourth, the apparent molecular sizes of the deoxycholate-solubilised HL-A antigens were smaller than those reported previously for intact HL-A and H-2 antigens solubilised in NP 40 (200,000 and 380,000 respectively[8,10]). Although the antigens may exist in the membrane as aggregates[10] which were dissociated by deoxycholate, this seems unlikely since deoxycholate failed to cause any detectable dissociation of non-covalently bonded, multi-subunit proteins such as aldolase and haemoglobin (M. J. Crumpton and M. J. H. in preparation). The difference is probably related to the different properties of the detergents (such as micelle size) and possibly expresses different extents of binding. If this interpretation is correct then the functional membrane unit of H-2 and HL-A antigens may be a non-covalently bonded dimer with a molecular weight of about 90,000. The partially purified material has been used for the preparation of antisera in C_3H and Swiss-Webster mice. Preliminary results indicate that cytotoxic antisera can be made against the glycoproteins but not against the proteins from the BRI 8 membranes, and suggest that this material may prove very useful for raising HL-A antisera.

This study incorporates four significant observations. First HL-A antigens are located mainly, if not exclusively, in the cell surface membrane. Secondly, all the plasma membrane HL-A antigens are glycosylated and may be separated from the non-glycosylated proteins by affinity chromatography of deoxycholate-solubilised membrane on a column of LcH-Sepharose. Third, the molecular size of the series 4 antigens in deoxycholate is smaller than that of the LA series. Fourth, the intact HL-A antigens are larger than the papain-solubilised fragments. Although the actual molecular size of the membrane-bound antigens is at present uncertain, some results suggest it is about 90,000.

The methods used in this study have avoided the technical difficulties associated with the isolation of water-insoluble membrane proteins in a biologically active form and should enable the separation of the whole antigens in amounts sufficient for structural studies.

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Human Normoblast A Antigen seen by Immunoelectron Microscopy

MEMBRANE antigens are extensively studied on different mammalian cell lines. Antigens of the erythrocyte series are often chemically well defined heterosaccharide determinants[1], the subject of numerous studies, most of them restricted to the mature circulating erythrocytes. There are only a few reports dealing with the detection of blood group antigens on erythrocyte precursor cells[2–5]. Yunis and Yunis[2] gave strong evidence of the presence of A, B and H alloantigens on human normoblasts, mostly by indirect agglutination methods involving living bone marrow cells.

Here we report immunoelectron microscopy observation of A antigen on human normoblasts using peroxidase-labelled antibodies which allow detection of surface antigens on separated cells. A preliminary report has appeared[6] in which A antigen was detected by this method on circulating normal erythrocytes; a heavy labelling of A_1 red cells was found in most cases, and A_2 cells exhibited marked variations of antibody binding capacity.

Bone marrow was obtained by iliac puncture from four normal individuals (three A_1, one A_2). Aspirates were collected in Hank's heparinised solution (Liquemine Roche) and bone marrow particles isolated and dissociated by pipetting in Hank's. Nucleated cells were separated from fat droplets by several washes and centrifugation for 5 min at 200*g*. They were then fixed with phosphate-buffered glutaraldehyde (T.A.A.B. Laboratories) 1.25% for 30 min in the cold, and kept in buffer until following steps.

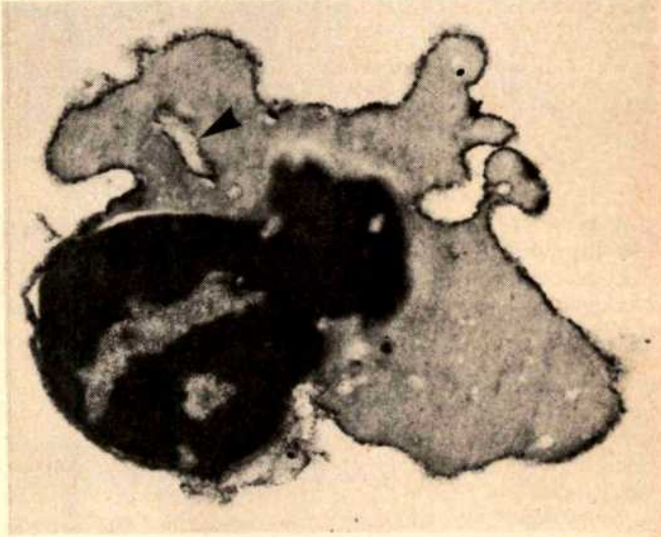

Fig. 1 Orthochromatic normoblast with nucleus in the way of expulsion. This motile cells has irregular borders, with a continuous labelling including the thin perinuclear cytoplasmic corona. Arrow, cross section of a membrane invagination, also labelled. Staining with uranyl acetate and lead citrate (×7,600).

The antiserum used was a human alloantiserum with an agglutination titre of 1:256 of A_1 cells which decreased to 1:2 after adsorption on A_2 cells. Activity was due mostly to IgM as the titre decreased to 1:8 after 2-mercaptoethanol treatment[7]. Monospecific anti-human IgM antibodies were prepared in rabbits. Purification was carried out by elution from IgM and cross reaction with IgG solid immunoadsorbents[8]. Human IgM and IgG were purified by previously described methods[9,10]. Purified rabbit anti-human IgM antibodies were conjugated with horse radish peroxidase (Sigma Laboratories) by a two step method[11].

Fixed cells were mixed with anti-A serum (3% cell suspension in 1:2 serum dilution) for 90 min at room temperature, extensively washed, and fixed again with glutaraldehyde for 10 min. After washing, sensitised cells were incubated with anti-IgM antibodies (1 mg ml^{-1}) for 1 h, washed and briefly fixed. Immunocytochemical staining was carried out by a 30 min incubation in the usual mixture of DAB and H_2O_2 [12]. Cells were then post-fixed with a 1% osmium tetroxide, dehydrated and embedded in Epon.

Specificity of labelling was ensured by the following controls: the treatment of group O and B bone-marrow cells; the omission of anti-A antibodies; the selective inhibition of labelling by non-conjugated anti-IgM; the use of normal conjugated rabbit IgG instead of anti-IgM and the incuba-

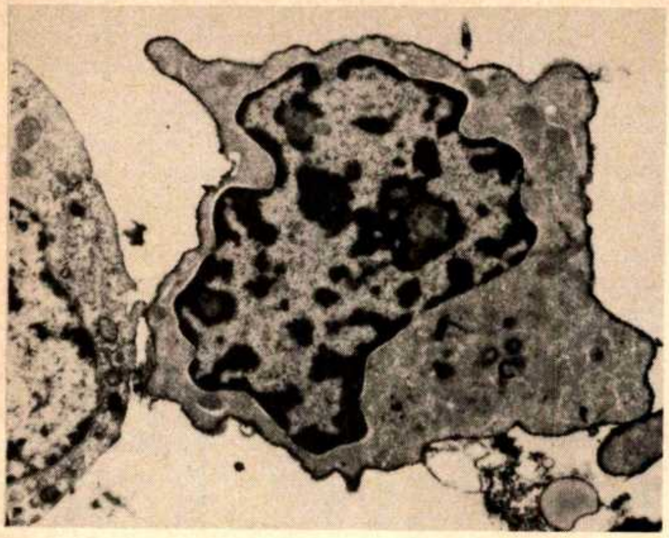

Fig. 2 Basophilic normoblast. The nucleus contains small clumps of heterochromatin. c, Two centrioles. The contiguous granulocyte precursor is not labelled. Staining with uranyl acetate and lead citrate (× 7,600).

tion of cells with peroxidase alone.

A dense and continuous dark labelling was seen in contact with the membrane of all normoblasts, surrounding the whole perimeter of the cell, including rhopheocytosis invaginations. No significant variations of the thickness of the dark layer could be seen among normoblasts of a single bone marrow. A_2 normoblasts could exhibit slight variations in the labelling intensity, however, which had no relation to the maturation stage. Precise identification of the differentiation stage was made according to nuclear and cytoplasmic ultrastructural features[13]. Thus, all recognisable stages including pronormoblasts seemed to have a high density of antigen. Recognition of pronormoblasts may be difficult even at the ultrastructural level[14] but the presence of ferritin-containing vesicles resulting from rhopheocytosis, revealed by high power views of these labelled undifferentiated blast cells, allowed a firm identification.

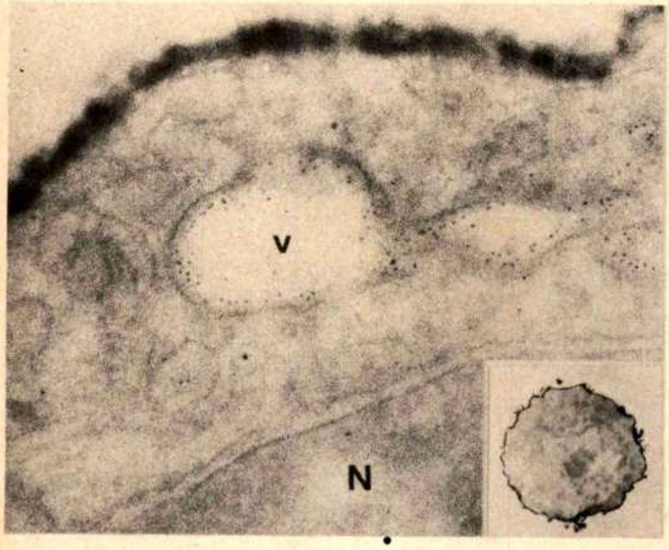

Fig. 3 Inset, heavily labelled pronormoblast. Large nucleolus is visible (× 2,300). High magnification showing the membrane labelling, and ferritin molecules in micropinocytotic vesicles (v); these rhopheocytosis vesicles result from invagination of the outer membrane, previous to treatment of the cells and thus are not labelled. N, Nucleus (× 91,500).

Occasionally a thin and limited labelling of the membrane of granulocytic and monocytic cells could be seen, sometimes in close contact with labelled normoblasts. Although the possibility of a 'false' labelling of these contiguous cells by 'flowing' of the reaction product, as already discussed by others[15], cannot be excluded, this seems improbable as many other contiguous cells did not exhibit labelling at all. Finally, this faint positive reaction could result from the presence of A antigen on these non-erythrocytic cells, as already indicated by other methods[16], but this requires further investigation. In addition, some lymphocytes and plasma cells were labelled and this was presumably due to the detection of surface IgM, as they were found in controls when anti-A was omitted as well as when O and B cells were tested.

The labelling of normoblasts appeared as a continuous layer of dense product, as in circulating erythrocytes[6]. No discontinuity was observed, suggesting a periodic pattern of A sites, and this is at variance with other studies using ferritin-labelled antibodies[5,17]. Reasons for these discrepancies may be of a technical nature, as discussed elsewhere[18].

Thus, our observations confirm and extend previous data from Yunis and Yunis[2] by demonstrating at the ultrastructural level the presence of A antigen on erythrocyte precursor cells. Indirect evidence of the presence of erythrocyte antigens on early precursors was also reported in mice[3]. On the other hand, some data on animals indicates that the appearance of antigen sites is in relation to the maturation of normoblasts, with detectable and reactive sites at later stages of this process[4,5]. Although not quantitative, our findings clearly show, at least for human alloantigens, that such receptors are already numerous at the very early stage of erythrocyte differentiation.

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Intracellular Location of Specific Antibodies Reacting with Human Lymphocytes

THE concept of the continuous renewal of the plasma membrane of a cell has been well supported by studies on the rate at which histocompatibility antigens on the surface of the cell are replaced[1-4]. Thus, if HL-A or H-2 antigens are removed by treatment with proteolytic enzymes, about 6 h elapse before the cell resynthesises them and they are again detectable with the appropriate antisera. When lymphocytes are incubated with a labelled anti-HL-A serum, label is gradually released during the next 6–8 h and the complement-dependent cytotoxic effects of an anti-HL-A serum are lost in a similar way; we call this 'escape from sensitisation'. This suggests that the turnover of the membrane of a cell may be primarily responsible for the ability of the cell to deal with antibodies binding to it, but the mechanisms involved are by no means clear. It has been suggested that antibody could either be released from the surface of the cell as an antigen-antibody complex or that it might be internalised, catabolised and then released, but neither of these ideas has been fully investigated. The early fate of fluorescent material has however been extensively studied[5,6,7].

Some subcellular fractions of the cell are known to carry the same type of histocompatibility antigens as those found on the plasma membrane[8-11], but it is not known whether there is any relationship between these internal antigens and those on the surface of the cell or whether they play any part in the way in which the cell deals with anti-HL-A antibodies. By following the fate of ^{125}I-labelled antibodies we thought that it might be possible to study these problems and we have therefore attempted to examine both the role of these internal antigens and the fate of the antigen-antibody complex formed at the cell surface.

Monospecific HL-A2 and HL-A8 antisera obtained from patients with choriocarcinoma and an antilymphocyte serum which was obtained by repeated injection of a rat with human lymphocytes were used, and were labelled in the manner described by Hunter and Greenwood[12].

The results of the two different methods for measuring the loss of antibodies are compared in Fig. 1. Lymphocytes from an HL-A2 individual were sensitised by incubating them at 37° C for a short period (30 min) with a limited amount of an HL-A2 antiserum. After washing the cells to remove excess antibody, the complement dependent lytic activity was determined at hourly intervals. Lymphocytes carrying the same histocompatibility antigen were treated in a similar way with ^{125}I-labelled antiserum so that the rate of loss of label could be compared with the rate at which the cytotoxic effects of antibody disappeared. Despite the fact that the two curves are very similar, it is evident that when all the cytotoxic activity of the serum has been lost, there is still a proportion of labelled material which is retained by the cells.

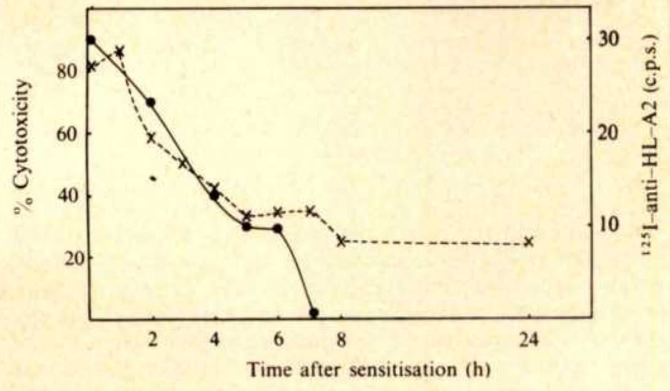

Fig. 1 Rate of loss of labelled anti-HL-A2 antibody (× . . . ×) and loss of complement-dependent cytotoxicity (●—●) from lymphocytes of an HL-A2 individual. Antiserum was labelled in the manner described by Hunter and Greenwood[12], and cells were maintained in Eagle's MEM + 10% foetal calf serum after a 30 min sensitisation. Complement-dependent cytotoxicity was determined in the way described by Miyajima *et al.*[4] except that sensitisation was carried out at 37° C.

Table 1 Amount of ^{125}I-labelled anti-HL-A2 antibody binding to equal numbers of cells or nuclei * at 37° C

	c.p.s. HL-A2 individual	Non-HL-A2 individual
Cells	299.4 ± 45.2	20.2 ± 10.9
Nuclei	2,303.8 ± 99.3	4.4 ± 0.9

* 2×10^6 ml^{-1}.

The results of autoradiography and cell fractionation show that this residual antibody is no longer associated with the plasma membrane and escape seems to involve the intracellular relocation of antibody or antigen-antibody complex. This might account for the apparent inactivity of antibody retained by the cell, for once it has been removed from the plasma membrane complement-dependent cytotoxicity is no longer expressed.

Figure 2 shows that the labelled antibody which binds to the surface of cells is first processed through the patching and capping mechanism which has now become well established, but the step which follows this internalisation leads to the deposition of antibody in or around the nucleus.

Cell fractionation studies, Fig. 3, confirmed these observations and they also showed that label is lost from the cytoplasmic

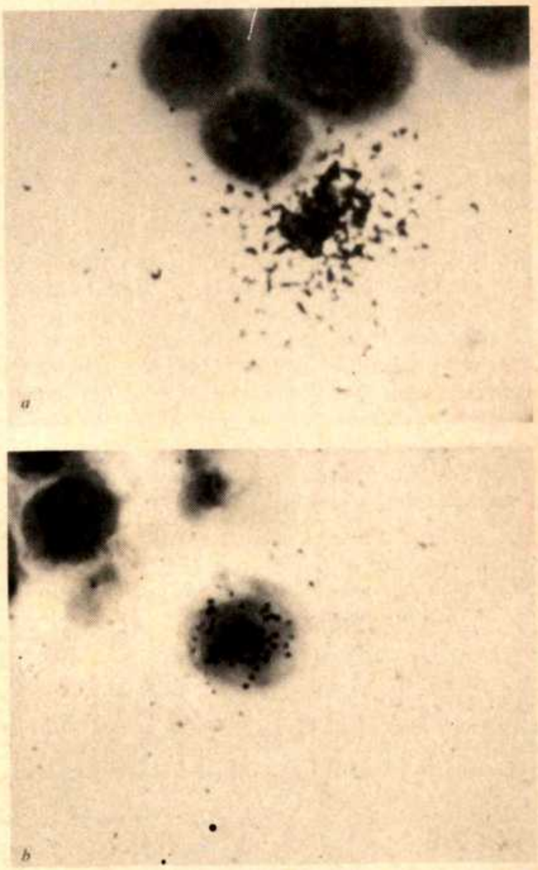

Fig. 2 Autoradiography of cells treated with ^{125}I-HL-A2. *a*, Cells immediately after sensitisation; *b*, 24 h after sensitisation.

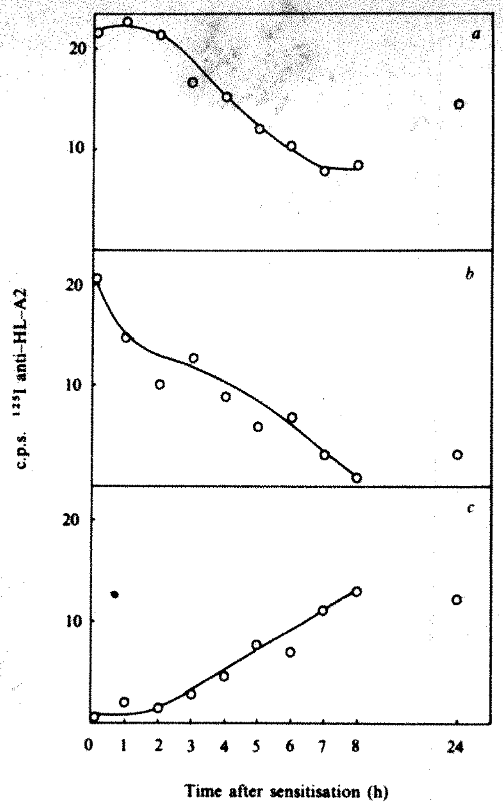

Fig. 3 ^{125}I-anti-HL-A2 in various cell fractions at intervals after sensitisation. *a*, Cells; *b*, cytoplasmic and, *c*, nuclear fractions prepared in the way described by Pegrum *et al.*[13], cytoplasmic fractions being collected as washings after deoxycholate and Nonidet P40 treatment.

fraction at around 8 h. Escape from complement-dependent sensitisation therefore seems to be complete when antibody has been removed from the cytoplasm of the cell.

Albert and Davies[11] have shown that the nuclear membranes of cells carry the same histocompatibility antigens as those found on the external membrane. We also found that nuclear preparations carry HL-A antigens, for ^{125}I-anti-HL-A binds both to isolated lymphocyte nuclei and to whole cells. In fact, from the results shown in Table 1, it seems that nuclear preparations have a higher affinity for anti-HL-A antibodies than intact cells. This might suggest that the label we found in the nucleus after cell fractionation is the result of uptake during the extraction process. The autoradiographs, however, provide convincing evidence that antibodies are present in the different regions of the cell during the course of escape. We also observed that label could only be detected in the cytoplasmic fraction during the first few hours after sensitisation when the amount of antiserum binding to the cell was at its highest.

Table 2 Amounts of ^{125}I-labelled anti-HL-A8 antibody in various cell fractions at daily intervals after sensitisation at 37° C

	c.p.m.				
	Initial	24 h	48 h	72 h	96 h
Cells	1,097.5	492.0	681.0	549.3	620.5
Cytoplasm	1,220.3	60.6	126.9	80.7	9.75
Nuclei	120.7	626.3	672.0	693.2	645.2

In theory, it should be possible to determine the rate of turnover of HL-A antigens in the nucleus or on the nuclear membrane by following the fate of the ^{125}I localised in this region. The amounts in the various cell fractions were therefore assessed at daily intervals for some time after most of the antibody had been lost during escape and the results of the experiment are shown in Table 2. Even after 4 d, the amount of labelled material in the nuclear fraction was at a relatively high level, the values being practically the same as those obtained from cells which had recently escaped sensitisation. Although this suggested that the nuclear membrane was very stable the same kind of results were not always obtained when the experiments were repeated with an anti-lymphocyte serum (ALS). We found that label only remained in the nucleus if the amount of antiserum used to treat the cells was kept to very low levels. The addition of anti-lymphocyte serum in quantities high enough to induce blastogenesis caused label to be released from both nucleus and the whole cell at a time which coincided with DNA synthesis; that is at about 72 h (Fig. 4). It is known that anti-HL-A serum is also capable of stimulating mitosis in lymphocytes and when cells were maintained in medium containing high levels of an anti-HL-A8 DNA synthesis was initiated. By first treating cells with labelled serum and then incubating them in medium containing greater amounts of the same cold antiserum, it could be shown that anti-HL-A is dealt with in a similar way to ALS. As Table 3 shows, when lymphocytes are stimulated with high levels of anti-HL-A any ^{125}I-antibody already associated with the nucleus is released.

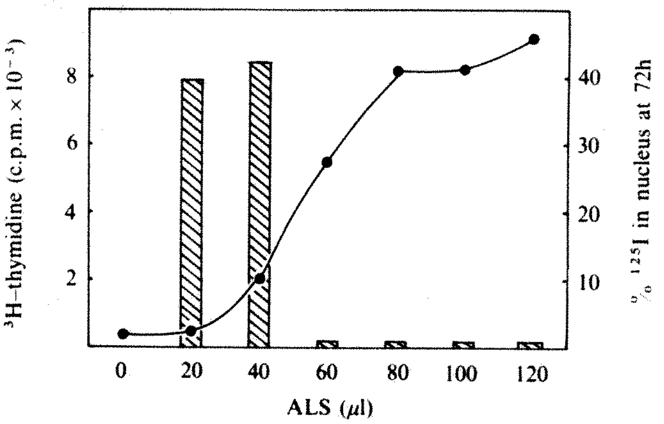

Fig. 4 DNA synthesis at 72 h in lymphocytes treated with various quantities of anti-lymphocyte serum (ALS). 10^6 cells ml^{-1} were maintained at 37° C in Eagles MEM+10% foetal calf serum containing 0–120 μl ml^{-1} ALS. DNA synthesis was assessed over a 16 h period at the end of culture by the addition of 2.5 μCi ml^{-1} ^{3}H-thymidine. The amount of ^{125}I-ALS in the nucleus was determined as described in the legend to Fig. 3. 6×10^7 lymphocytes were treated for 30 min at 37° C with 0.1 ml of labelled serum. After washing the cells were distributed into vials containing medium with the various concentrations of ALS shown in the figure. Cell fractionation was carried out at 72 h.

Some idea of the mechanism which leads to the stimulation of lymphocytes in this experiment can be obtained by repeatedly treating cells with antiserum (Fig. 5). One day after the first sensitisation all complement dependent cytotoxicity was lost although ^{125}I-anti-HL-A2 was retained in the nucleus. After escaping from a primary challenge, cells could be resensitised by treating them a second time and a second escape cycle then took place. Parallel experiments using labelled antiserum showed that the second treatment initially serves to supplement the amount of label which remains after the first escape. When the second cycle of escape is complete, however, the total amount of labelled antibody in the cell is much greater than after the first cycle, and cell fractionation shows that the additional label is accumulated by the nucleus.

This amassing of antibody or a catabolised form of antibody in or around the nucleus therefore seems to be a step which is essential for lymphocyte triggering and although we do not yet know what processes are involved in this nuclear localisation it is thought that antibody is able to function as a derepressing agent at this site. With low levels of antiserum there is no

Table 3 Amounts of ^{125}I-labelled anti-HL-A8 in the various cell fractions during stimulation of cells with antiserum

	c.p.m.			
	Initial	24 h	48 h	72 h
Cells	1,749.2	735	756	53.1
Cytoplasm	1,839.8	10.6	10.8	29.7
Nucleus	14.9	699.0	761.1	47.6
DNA synthesis ^{3}H-thymidine c.p.m./10^5 cells	822.9	475.5	563.7	1,077.0

Cells were sensitised with ^{125}I-labelled antiserum for 30 min at 37° C, washed, and incubated in Eagle's MEM containing 70 µl ml^{-1} of cold anti-HL-A serum. DNA synthesis was estimated in separate cultures as described above.

stimulation of DNA synthesis and it is probable that lymphocyte mitotic activity is initiated only when the levels in the nucleus reach a certain threshold. Although it has been suggested that the patching and capping process which follows the treatment of cells with an antiserum is a prerequisite for lymphocyte triggering[5], our results suggest that additional nuclear events are equally important.

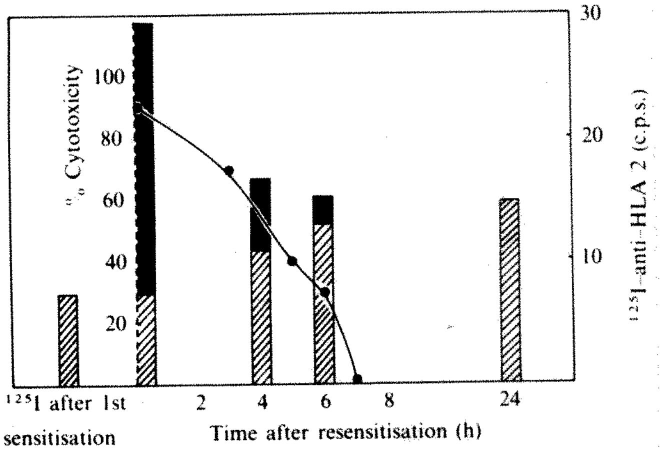

Fig. 5 Resensitisation of lymphocytes with an anti HL-A2 serum (the same quantity as for the first sensitisation). Complement-dependent cytotoxicity and cellular distribution of ^{125}I-anti HL-A were assessed; ▨, amounts in the nucleus; ■, amounts in the remainder of the cell.

There is already some evidence that phytohaemagglutinin and other mitogens may be localised in the nucleus prior to lymphocyte stimulation[14–16] and although it has been shown that mitotic activity can be initiated without the mitogen entering the cell this only happens when mitogens have been complexed in some way[17,18].

Our results show that the cell is capable of internalising anti-HL-A and antilymphocyte antibodies in much the same way as it internalises mitogens, and this seems to be necessary for this type of lymphocyte stimulation. We consider that the nucleus of a cell may play an equally important role in immunological events as changes which take place at the surface of the cell.

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Survival of Unmodified Spleen Allografts in Rats

Laboratory models which provide for reproducible acceptance of organ allografts with neither preoperative conditioning or postoperative immunosuppression of recipient animals are rare. In pigs, however, liver allografts are generally accepted even if histocompatibility differences are strong[1–3]; and indefinite acceptance of renal allografts in rats across major Ag-B barriers has been described[4].

I have described the fate of spleen allografts between PVG and AGUS rats[5]. Grafts were rejected within 3 weeks but survived indefinitely when recipients were injected with cells from the donor strain before spleen grafting. Subsequent PVG skin grafts to spleen-bearing rats survived indefinitely when given 6 weeks or later after spleen grafting. Third party skin grafts were rejected as in controls. When long surviving spleen grafts were removed, recipients continued to accept further PVG skin allografts.

I hypothesised that the pretreatment had mitigated host-*versus*-graft (HvG) and supported a more enduring graft-*versus*-host (GvH) activity. I therefore felt that spleen allografts might survive without any recipient conditioning where donor–recipient reactivity was more balanced.

I describe here spleen allografts in inbred rats across strong Ag-B barriers without pretreatment or postoperative immunosuppression. Skin allograft survival was used to monitor recipient response towards donor and third-party tissue.

Three inbred rat strains were used: Wistar rats (Ag-B 1), AGUS rats (Ag-B 2), and PVG rats (Ag-B 5), obtained from Medical Research Council Laboratory Animals Centre, Carshalton, Surrey. Male or virgin female rats weighing approximately 200 g served as donors and recipients. In the recipient operation the spleen with an aortic cuff bearing the coeliac axis, and the portal vein were anastomosed end-to-side to the abdominal aorta and vena cava[5]. The recipient's own spleen was not removed. Full-thickness skin grafts with areas of 2×3 cm were sewn to graft beds on the back and covered for 7 d.

Criteria for spleen survival were both macroscopic and histological appearances at repeated laparotomies. The criterion for skin graft survival was hair growth. Rats dying within 2 weeks of operation through technical failure were excluded from this report. None of the excluded rats showed signs of lethal GvH disease.

Table 1 Spleen allograft survival

Group	No. of animals	Donor	Recipient	Graft survival
1	22	PVG	AGUS	2–3 week
2	13	AGUS	PVG	7–8 month*
3	25	Wistar	AGUS	6–10 month*
4	11	AGUS	Wistar	3 month*

* Animals are still alive with viable spleen grafts.

Spleen grafts from PVG to AGUS rats were rejected within 3 weeks, whereas spleen grafts from AGUS to PVG, Wistar to AGUS and AGUS to Wistar rats are without exception still viable up to 10 months after grafting (Table 1). The grafts grew to between three to six times normal size. Maximum size was reached after about 3 weeks. Then instead of being rejected, as the spleens of group 1, the grafts remained viable with a good circulation and showed a tendency to reduce slowly in size. They were fairly soft in consistency, whereas rejecting grafts became swollen, hard, and patchy, then white and necrotic, and were finally absorbed completely. Histological findings of surviving spleens in groups 2 to 4, and the recipients' own spleens did not differ from those described recently[5].

Table 2 Skin allografts to rats of Table 1 carrying spleen allografts

Group	No. of animals	Donor	Recipient	Time between spleen and skin grafting	Graft survival
1	8	PVG	AGUS	2 months	8.4±0.5 d
2	7	AGUS	PVG	4 months	3 months*
3	7	Wistar	Agus	4 months	4–5 months*

* Animals are still alive with viable spleen and skin grafts.
Animals of group 4 have not yet been skin grafted.

Skin grafts reflected the fate of the spleen grafts: skin grafts to rats of group 1, where the spleen grafts had been rejected, were rejected like controls, whilst skin grafts are still viable three to five months later after grafting on rats of groups 2 to 3 (Table 2). Spleen grafted rats rejected third-party skin allografts (Table 3). In all four groups, control skin allografts were rapidly rejected (Table 4).

It was demonstrated that the rat spleen allograft can be accepted and then induce a donor-specific tolerance to subsequent skin allografts. Some of the questions arising were: (1) were the recipients peculiar from an immunological point of view? (2) was the grafted organ lacking antigenicity? (3) to what extent was spleen graft survival dependent on immunological competence and competition of both graft and host?

Table 3 Third party skin allografts to rats of Table 1 carrying spleen allografts

Group	No. of animals	Donor	Recipient	Time between spleen and skin grafting	Graft survival
1	6	Wistar	AGUS	4 months	9.1±0.4 d
2	7	Wistar	PVG	4 months	7.6±0.8 d
3	6	PVG	AGUS	4 months	8.1±0.3 d

In answer to question (1), all three rat strains behaved as if immunologically normal. They rapidly rejected control skin grafts. Heterotopic cardiac allografts exchanged between these rats were quickly rejected[6]. There were good responses in MLC between the strains used.

In answer to question (2), the spleen grafts were recognised as foreign by recipients in all combinations. In group 1 they were rejected. In groups 2 to 4 they survived initial HvG. Histological signs of early graft damage were apparent but finally a state of equilibrium seemed to have been reached with maintenance of the circulation and viability of the tissue.

Table 4 Control skin allografts in strain combinations used for spleen grafting

Group	No. of animals	Donor	Recipient	Graft survival
1	9	PVG	AGUS	7.7±0.3 d
2	7	AGUS	PVG	8.8±0.6 d
3	6	Wistar	AGUS	9.4±0.7 d
4	7	AGUS	Wistar	8.2±0.4 d

In answer to question (3), a basic requirement for spleen graft survival seemed to be immunological competence and undisturbed competition of graft and host. Two results supported this: first, spleen allografts from AGUS rats to sublethally irradiated PVG recipients induced lethal GvH disease; second, spleen allografts from sublethally irradiated AGUS donors to PVG rats were promptly rejected (unpublished data). This implied that spleen allografts resulted in a confrontation of two immunocompetent systems. Their interaction might determine the rather puzzling outcome of graft survival. Interference with this interaction of graft and host was fatal for one side.

Follow-up skin grafts showed that rats with a viable spleen allograft were rendered specifically unresponsive towards the spleen donating strain, but not other strains. The spleen seemed to be a non-vital organ but it may prove to play a role in processes leading to allograft acceptance.

Immunosuppression in clinical transplantation is entirely aimed at inhibiting host action against the graft. But it prevents, as a side effect, graft-*versus*-host action as well. More knowledge about the mechanisms leading to the results described might initiate a second look at current treatment after transplantation of organs. It might be possible to encourage, instead of inhibiting graft and host reactivities, having in mind their balanced immunological interaction and not paralysis.

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Sensitivity to Amphotericin B of *in vitro* Established Cell Lines

IT has been demonstrated that the antifungal lipophylic polyene amphotericin B inhibits eukaryotic cell growth by binding to sterols and making 'holes' in cell membranes[1]. Used at subinhibitory concentrations, it enhances penetration of other antibiotics into the cells of higher organisms *in vitro* and *in vivo*, showing a synergistic effect with a number of unrelated drugs[1,2]. It has also been demonstrated that higher doses of amphotericin B inhibit macromolecular syntheses, probably by severely damaging the cell membrane[3].

Table 1 Cell lines used

Cell line	Origin	Characteristics	Reference
3T3	Mouse		10
3T3-TK$^-$	Mouse	3T3, thymidine kinase deficient	11
3TP	Mouse	3T3-TK$^-$, puromicin resistant	12
3T3-SV	Mouse	3T3, transformed by SV40	13
BHK-B1	Syrian hamster	BHK, thymidine kinase deficient	14
BHK-T6	Syrian hamster	BHK, hypoxanthine guanine phospho-ribotransferase (HGPRT) deficient	15
WG3	Chinese hamster	DON, HGPRT deficient	16
K455	Human	HGPRT, deficient, primary explant (Lesh-Nyhan)	Personal communication (from Klinger through Siniscalco)

We report here our investigations on the sensitivity to amphotericin B of several *in vitro* established cell lines, and of hybrid clones derived from two lines which exhibited very different degrees of sensitivity. We observed a positive correlation between levels of density-dependent inhibition of cell growth and sensitivity to the drug.

A number of cell lines (Table 1) was tested for sensitivity to amphotericin B according to the method described by Medoff *et al.*[4]. This is based on the incorporation of ^{3}H-uridine into acid-precipitable material during an 18 h incubation of actively growing cells in Dulbecco's modified Eagle medium (GIBCO), supplemented with 10% foetal calf serum (Gray) and in the presence of increasing concentrations of the drug (Fungizone GIBCO). Figure 1 shows that some cell lines were extremely sensitive to the drug (3T3, 3T3-TK$^-$, K455), some were highly resistant (3T3-SV, BHK-T6, WG3), while others had an intermediate sensitivity (3TP, BHK-B1). When ordering these lines with regard to their sensitivity to amphotericin B (Table 2), we observed that the more sensitive ones were those showing a more severe contact inhibition of cell growth, as evaluated by the correlation with their efficiency of plating (EOP) in agar[5] and saturation density[6], despite the high variability in cell size of the various lines used. The finding that SV40-transformed 3T3 cells showed a very high degree of resistance was consistent with this observation.

Table 2 Amphotericin B sensitivity, saturation density and efficiency of plating in agar of cell lines used

Inhibitory concentration of amphotericin B* (μg ml^{-1})	^{3}H-uridine incorporation at 60 μg ml^{-1} of amphotericin B (% of control)	Cell line	Saturation density (× 10^4 cells cm^{-2})	EOP in agar
2–5	2	3T3-TK$^-$	4.2	$<10^{-5}$
2–5	4	K455	4	$<10^{-5}$
5–10	1	3T3	4.5	$<10^{-5}$
10–20	9	3TP	30	ND
40–60	48	BHK-B1	48	3×10^{-4}
>60	100	WG3	60	6×10^{-4}
>60	100	3T3-SV	35	7×10^{-2}
>60	100	BHK-T6	61	4×10^{-4}

* Concentration of amphotericin B which inhibits the incorporation of ^{3}H-uridine to 50% of control.

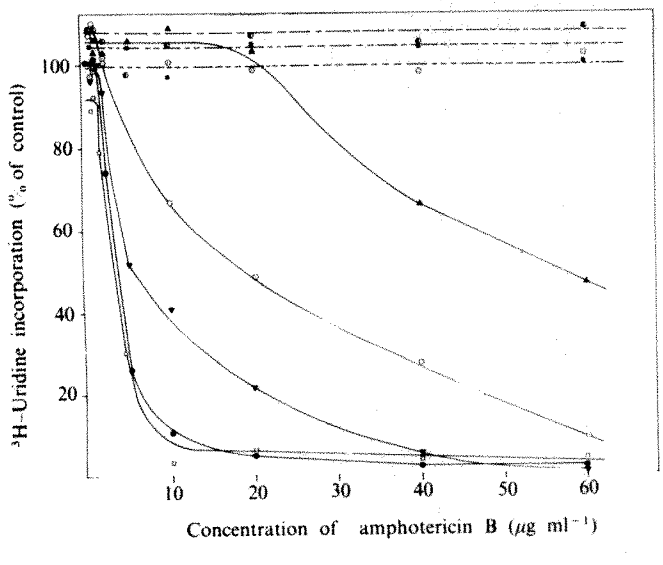

Fig. 1 Effect of amphotericin B on the incorporation of ^{3}H-uridine into TCA-precipitable material of various cell lines. ◐— - —◐, BHK-T6; ■— - - -—■, 3T3-SV; ○— - - - -—○, WG3; ▲—▲, BHK-B1; ○—○, 3TP; ▼—▼, 3T3; ●—●, 3T3-TK$^-$; □—□, K455.

To better analyse the observed correlation, we selected from 3T3-TK$^-$ cells (a highly contact-inhibited line) three clones capable of growing in agar by forcing growth beyond confluence for several passages. These three clones were tested for their sensitivity to amphotericin B (Fig. 2), and all showed a significantly increased resistance, as compared with the parental line. In addition, all the clones showed higher saturation densities than the parental line (Table 3).

Table 3 Characteristics of three sublines of 3T3-TK$^-$ selected in agar medium

Subclone	Inhibitory concentration of amphotericin B* (μg ml^{-1})	^{3}H-uridine incorporation at 60 μg ml^{-1} of amphotericin B (% of control)	Saturation density (× 10^4 cells cm^{-2})
KW-1	~10	32	19
KW-2	~10	34	18
KW-4	~10	28	21
3T3-TK$^-$	2–5	2	4.2

* Measured as in Table 2.

When a rare survivor from 3T3-TK$^-$ cells treated with 20 μg ml^{-1} of amphotericin B was tested for its ability to grow in agar, or its saturation density was measured, we again observed a correlation between resistance to the drug and release from contact inhibition of growth measured as saturation density (25 × 10^4 cells cm^{-2}).

To study the behaviour of amphotericin B sensitivity in cell hybrids between lines with extreme degrees of sensitivity, we selected spontaneous cell hybrids from BHK-T6 and 3T3-TK$^-$ cells by Littlefield's method[7] with techniques already described[8].

Twelve hybrid clones were selected and tested for their sensitivity to amphotericin B, saturation density and chromosomal constitution. Results are shown in Table 4. Judging by the shape of inhibition curves shown in Fig. 3, characterised by an initial strong decrease of ^{3}H-uridine incorporation similar to that of the 3T3-TK$^-$ parent and after an intermediate sensitivity between the two parental lines, hybrid lines may be composed of mixtures of cells exhibiting essentially two different levels of sensitivity to the drug.

Since mouse chromosomes are easily lost from hamster–mouse hybrids[9], it seemed possible that the observed levels of

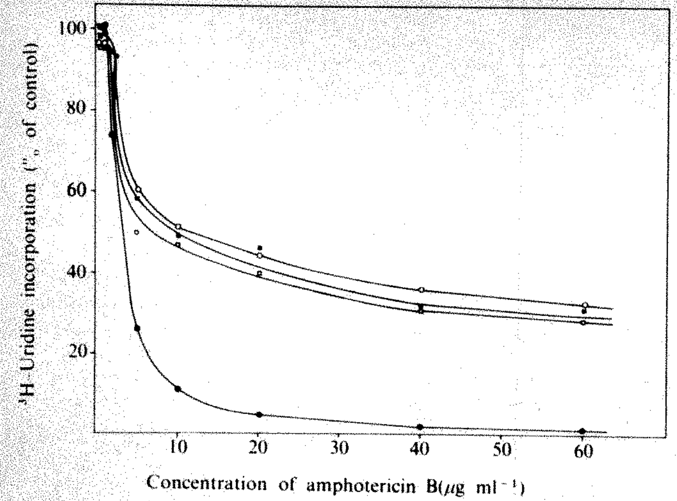

Fig. 2 Effect of amphotericin B on the incorporation of ^{3}H-uridine into TCA-precipitable material of clones derives from 3T3-TK⁻ cells. ○—○, KW1; □—□, KW2; ■—■, KW4; ●—●, 3T3-TK⁻.

Table 4 Levels of sensitivity to amphotericin B, saturation density and chromosomal constitution of BHK-T6 × 3T3-TK⁻ hybrid lines

Hybrid line	Inhibitory concentration of amphotericin B* (μg ml^{-1})	^{3}H-uridine incorporation at 60 μg ml^{-1} of amphotericin B (% of control)	Saturation density (× 10^4 cells cm^{-2})	Telocentric† / total
1	10–20	30	10	0.48
4	> 60	87	41	0.28
8	> 60	60	22	0.35
13	10–20	32	13	0.46
17	> 60	72	20	0.36
21	10–20	34	10	0.58
22	10–20	27	9	0.48
23	2–5	3	3.5	0.62
26	10–20	29	11	0.46
32	> 60	84	37	0.28
35	> 60	82	40	0.30
39	10–20	32	12	0.45

* Measured as in Table 2. See Fig. 4.

† Mean of at least ten karyotypes done after 3 week from hybridisation and at the time of amphotericin B sensitivity and saturation density determination. Telocentric chromosomes are mainly of mouse origin[9].

sensitivity to amphotericin B were correlated with different numbers of mouse chromosomes present in the hybrids. To test this possibility the hybrid line 21 exhibiting intermediate sensitivity was further subcultured in hypoxanthine–aminopterin–thymidine (HAT) medium for approximately one hundred generations. These cells were then resistant to high levels of amphotericin B (Fig. 3), their saturation density had increased to 35 × 10^4 cells cm^{-2}, and their chromosomal constitution had changed significantly (telocentric/total = 0.30).

The hybrid cell lines analysed exhibited an increased sensitivity to amphotericin B compared to hamster parent proportional to the ratio of the mouse chromosomes they carry (Table 4), thus showing dominance of the mouse sensitivity character over the hamster resistant one.

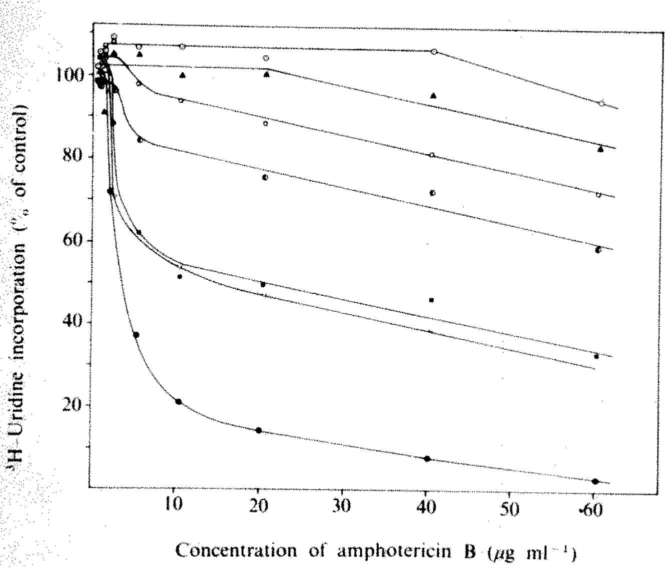

Fig. 3 Effect of amphotericin B on the incorporation of ^{3}H-uridine into TCA-precipitable material of some representative hybrid lines derived by fusion of BHK-T6 × 3T3-TK⁻ cells. ●—●, Hy 23; ▽—▽, Hy 1; ■—■, Hy 21; ◐—◐, Hy 8; □—□, Hy 17; ▲—▲, Hy 32; ⊙—⊙, Hy 21, after a hundred generations from first determination.

The drug sensitivities of the hybrid lines could be accounted for if the individual lines were mixed, containing various relative fractions of sensitive cells still carrying the relevant mouse chromosome and cells that have lost the chromosome and become resistant. It could also be that the lines are individually more or less pure and the drug inhibition pattern is gene dose dependent and characteristic of the multiplicity of the relevant mouse chromosomes present in the line. The segregation of resistant cells after further cultivation in HAT medium substantiates the proposal that the sensitivity derives from the presence of mouse chromosomes in the hybrid cell lines.

Since the continuous selection in HAT medium did not interfere with the segregation of hybrid cells resistant to amphotericin B, as in the case of hybrid line 21, it seems that the mouse allele responsible for sensitivity to the drug is not linked to the HGPRT allele.

Cells with reduced membrane-mediated control of growth (reduced levels of contact inhibition) exhibit increased levels of resistance to amphotericin B. Cells exhibiting increased levels of resistance to amphotericin B show reduced levels of contact inhibition. We conclude the action of amphotericin B on cell membranes is mediated through a membrane component involved in regulation of growth.

It is also apparent that amphotericin B, which is commonly used as an antifungal agent in cell culture media, imposes selection in favour of cells with decreased membrane-mediated growth control. We are examining the possibility that the use of amphotericin B may provide a convenient selective agent for virus-transformed cells.

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Increased Mobility and Redistribution of Concanavalin A Receptors on Cells infected with Newcastle Disease Virus

CONCANAVALIN A (con A) and various other plant lectins have been used extensively to study differences in the organisation of the surface of normal and transformed cells[1-3]. Cells transformed by oncogenic viruses are agglutinated by doses of con A which do not affect normal cells, but the latter can be made susceptible to agglutination by small doses of con A by brief trypsinisation. Furthermore, the susceptibility of normal cells to agglutination by con A can be increased by infection with various non-oncogenic enveloped viruses, including herpesviruses[4,5], poxviruses[6], arboviruses[7], paramyxoviruses[8,9] and rhabdoviruses[9]. The mechanism of this increased agglutinability is unknown. Since the glycoproteins in the envelope of such viruses can bind con A[9-11], their insertion into the plasma membrane of the infected cell during virus envelopment and release might increase the available con A receptors, which could account for the increased susceptibility to agglutination. We have detected no significant increase in con A receptors on cells infected with Newcastle disease virus (NDV) which were susceptible to agglutination by con A at concentrations that did not affect normal uninfected cells. We have found, however, that the increased agglutinability is due to a redistribution of con A receptors on the cell surface.

Baby hamster kidney (BHK) cells were cultured in Dulbecco's modified Eagle's medium in 60 mm plastic Petri dishes (Falcon) as described previously[12]. The origin and properties of the NDV strains[13-15], methods for infection of cell cultures[14,15] and measurement of the susceptibility of cells to agglutination by con A[4] have also been described. Con A was obtained as a twice crystallised protein (Miles-Yeda) and purified further by affinity chromatography[16]. Labelled con A was prepared from highly purified con A using ^{3}H-acetic anhydride[17]. ^{3}H-con A (specific activity 3×10^6 c.p.m. per mg con A) resembled native con A in its reactivity with rabbit anti-con A antiserum, co-chromatographed with unlabelled con A on DEAE-cellulose and agglutinated cells in doses similar to native con A. Binding of ^{3}H-con A to cells was measured at 0° C, 21° C and 37° C as described previously[18]. To calculate the specific binding of ^{3}H-con A, the amount bound in the presence of 0.1 M α-methylmannoside was subtracted from the amount bound in its absence. Kinetic analysis of ^{3}H-con A binding revealed saturation binding after 30 min of incubation at 0° C, 21° and 37° C.

The distribution of receptors on the cell surface was assessed by immunofluorescence using fluorescein-conjugated anti-con A antibodies[19-21], induced in rabbits by four subcutaneous injections of 2 mg purified con A in Freund's complete adjuvant followed by two intraperitoneal inoculations of 2 mg con A in alum. Rabbits were bled 1 week after the last injection, the anti-con A globulin-containing fraction was isolated from the serum and conjugated with fluorescein isothiocyanate[21,22]. Cells were exposed to con A at 0° C, 21° C and 37° C followed by further incubation at the appropriate temperature and then labelled with the fluorescein-conjugated anti-con A antibodies (F-anti-con A) for 1 h at the same temperature. Finally, the cells were fixed with 2.5% glutaraldehyde and the specific immunofluorescence pattern examined in a Leitz Ortholux microscope using ultraviolet illumination with a UG-1 excitation and K-430 barrier filter. As outlined below, in some experiments the cells were prefixed with glutaraldehyde before adding con A.

Measurements of ^{3}H-con A binding at 21° C or 37° C to BHK cells infected with virulent (Herts; Texas) mesogenic (Beaudette C) and avirulent (Queensland) strains of NDV revealed no significant difference from mock-infected cells (Fig. 1). Noonan and Burger[23] reported that measurements of con A binding made at room temperature or 37° C give misleading results and do not detect any significant differences in binding between cells. They proposed that true differences in con A binding to cell surfaces are obscured at these temperatures due to non-specific binding and endocytosis of bound con A. They reported that these problems were avoided if con A binding was measured at 0° C. However, measurement of ^{3}H-con A binding at 0° C to NDV-infected and uninfected BHK cells revealed that significantly less lectin was bound to cells than at higher temperatures but there was no significant difference in binding between infected and uninfected cells (Fig. 1).

Thus the increased agglutinability of NDV-infected cells does not seem to be due to an increase in available con A receptors; nor to changes in the size of cells after infection[18].

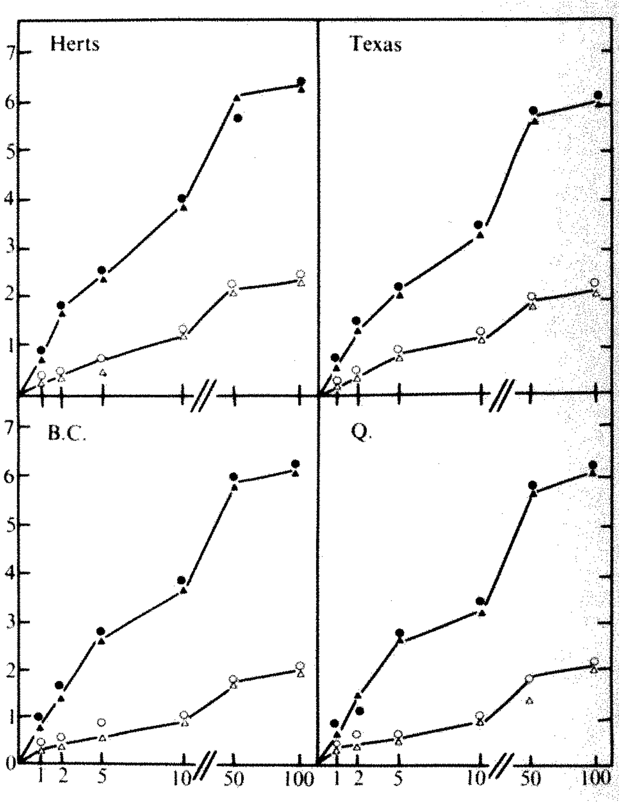

Fig. 1 Specific binding of ^{3}H-con A at 21° C and 37° C (closed symbols) or 0° C (open symbols) to BHK cells and BHK cells infected with NDV strains Herts, Texas, Beaudette C (B.C.) and Queensland (Q). ● and ○, infected cells; ▲ and △, uninfected cells. The results for binding of ^{3}H-con A at 21° C and 37° C were identical and for convenience are shown together. Binding measurements were made 7, 8 and 10 h after infection with strains Herts, Texas and B.C., respectively, at which times the cells were susceptible to agglutination by 500 μg con A ml^{-1} which failed to agglutinate uninfected control cells. In cells infected with strain Queensland binding was measured 16 h after infection.

It is possible, however, that the failure to detect an increase in con A receptors on NDV-infected cells, in spite of insertion of con A-binding viral proteins into the plasma membrane, is due to steric hindrance between adjacent con A molecules. For example, the con A receptors, including any new sites created by viral proteins, might be able to pack more closely than can the con A molecules themselves. This problem applies equally, however, to the numerous reports that significant differences in con A binding were not detected between cells that agglutinated and those that did not[1–3].

Our failure to detect an increase of receptors on NDV-infected cells agglutinated by con A resembles findings with virus-transformed and protease-treated cells[1–3]. With one exception[23], many studies have shown that the agglutination

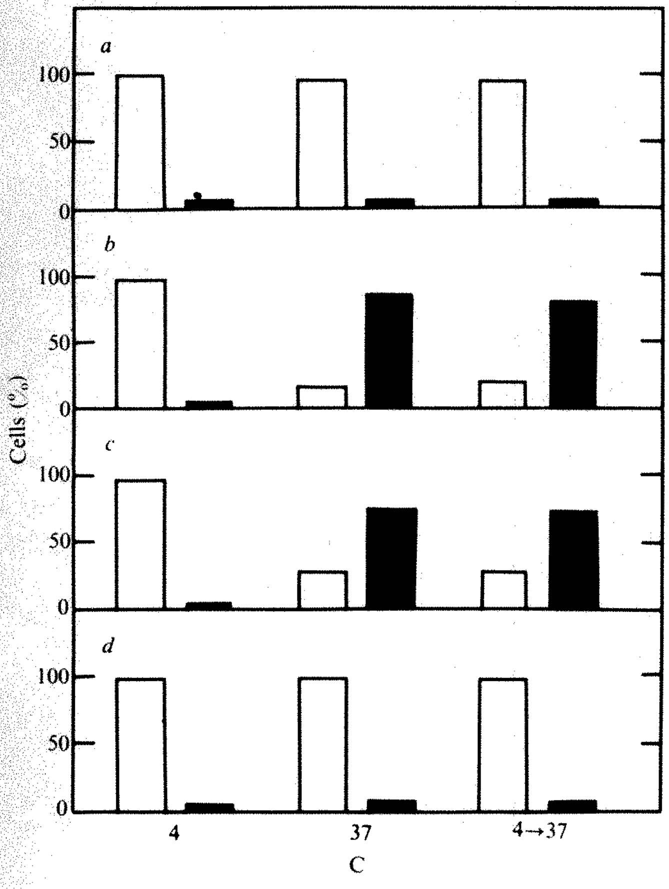

Fig. 2 The distribution of con A receptors on BHK cells detected by immunofluorescence using fluorescein-conjugated rabbit anti-con A antibodies (F-anti-con A) as described in the methods. Cells were examined after exposure to con A and F-anti-con A at 4° C, 37° C or exposure to con A at 4° C followed by warming and incubation at 37° C for 30 min before addition of F-anti-con A (4→37° C). The results are expressed as the percentage of cells showing uniform ring fluorescence (☐) characteristic of randomly dispersed con A receptors or showing 'patchy' fluorescence (■) characteristic of clustered con A receptors. The results are derived from three replicate experiments and a minimum of at least fifty cells were examined in each experiment. *a*, Receptor distribution at different temperatures on uninfected BHK cells exposed to con A and F-anti-con A before final fixation. *b*, Receptor distribution at different temperatures on BHK cells 1 h after treatment with 0.01% twice crystallised trypsin for 10 min at 37° C. The cells were treated with con A and F-anti-con A before final fixation. *c*, Receptor distribution at different temperatures on BHK cells 10 h after infection with NDV strain Beaudette C and exposed to con A and F-anti-con A before final fixation. • The cells were susceptible to agglutination by 500 μg con A ml^{-1} at 21° C or 37° C but uninfected cells were unaffected; *d*, receptor distribution at different temperatures on BHK cells 10 h after infection with NDV strain Beaudette C and fixed with 2.5% glutaraldehyde for 15 min at the appropriate temperature before exposure to con A and F-anti-con A.

of these cell types by small doses of con A is not due to an increase in con A receptors, but to their redistribution from a random pattern to form clusters[2,21,24,25]. Differences in the agglutinability of cells are presumed to result from differences in the organisation of the cell periphery which affect the ease with which receptors can move within the membrane to form clusters. We have investigated whether the increased agglutinability of BHK cells infected with certain NDV strains involved a similar redistribution of con A receptors.

We examined the immunofluorescence staining pattern on cells treated with con A and F-anti-con A before fixation to provide information on the inherent pattern of the receptors at a given temperature. Similarly, cells exposed to con A at 4° C and fixed without warming before addition of F-anti-con A provide information on the inherent topography of the receptor sites since their movement is reduced at low temperatures[21,24–26].

Uninfected cells treated with con A and F-anti-con A before fixation developed a uniform 'ring' immunofluorescence pattern with fluorescence confined to the periphery of the cell (Fig. 2*a*), characteristic of a random distribution of con A receptors[19–21]. 'Patchy' fluorescence staining, characteristic of aggregated or clustered con A receptors[19–21], was observed on less than 3% of cells at 4° C and 37° C (Fig. 2*a*). As a control to test the accuracy of the staining method, normal cells were also trypsinised to induce redistribution of con A receptors into clusters[19–21]. These cells, treated with con A at 4° C, showed predominantly the same uniform ring fluorescence pattern (Fig. 2*b*). Most trypsinised cells treated with con A at 37° C, or at 4° C followed by warming to 37° C, however, showed patchy fluorescence. The trypsinised cells also showed a maximum agglutination response to 500 μg con A ml^{-1} while non-trypsinised cells were unaffected.

Observations on the distribution of con A receptors on BHK cells infected with the Beaudette C strain of NDV, and which showed increased agglutinability, revealed uniform fluorescence on cells exposed to con A at 4° C, but most of the cells exposed at 37° C showed patchy fluorescence indicative of clustering of the receptors (Fig. 2*c*). Similarly, infected cells treated with con A at 4° C and warmed to 37° C before fixation showed predominantly patchy fluorescence (Fig. 2*c*). The change from predominantly uniform to predominantly patchy fluorescence after infection with Beaudette C occurred as the cells became increasingly susceptible to agglutination (Fig. 3*a*). Most cells infected with the avirulent NDV strain Queensland, which show no increased susceptibility to agglutination by con A compared with uninfected cells, revealed a consistent uniform fluorescence pattern throughout infection (Fig. 3*b*).

In contrast to the 'patchy' fluorescence found on most Beaudette C-infected cells treated with con A before fixation, staining of similar infected cells fixed with glutaraldehyde before addition of con A revealed uniform fluorescence (Fig. 2*d*). This indicates that the inherent topography of con A receptors on infected cells which show high agglutinability is no different from that on uninfected cells which fail to agglutinate. This suggests that the increased agglutinability of infected cells is not caused directly by virus-induced changes in the topography of con A receptors on the cell surface. Instead, the clustering of receptors is probably due to their greater mobility within the membrane, and is probably a secondary phenomenon induced by exposure to con A, presumably as a result of the cross-linking of adjacent receptors by tetravalent con A molecules. The mechanism by which infection with certain NDV strains could alter the cell surface to permit greater mobility of the con A receptors within the membrane is unknown.

These results reinforce the overall similarities between con A agglutination of normal cells infected with non-oncogenic viruses and the agglutination of transformed and protease-treated cells by similar doses of con A. For example, three studies have shown that neither transformation nor proteolytic modification of the cell surface lead directly to clustering of

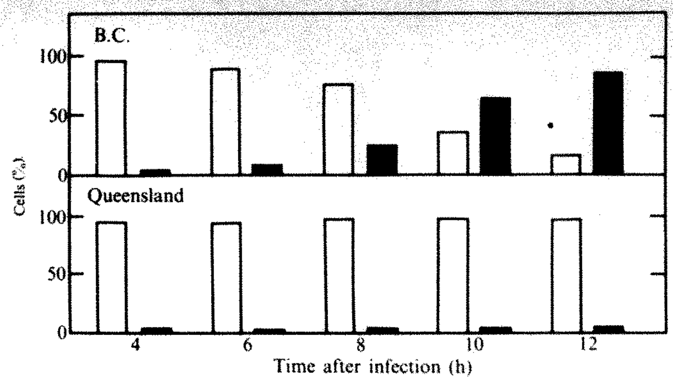

Fig. 3 The distribution of con A receptors on BHK cells at intervals after infection with NDV strains Beaudette C (B.C.) and Queensland. Immunofluorescence measurements were made on cells exposed to con A and F-anti-con A at 37° C before final fixation and the results determined as in Fig. 2. □, Uniform fluorescence and ■, patchy fluorescence. Cells infected with strain B.C. showed an increased susceptibility to agglutination by 500 μg con A ml^{-1} from 8 h after infection (+) and by 12 h after infection more than 90% of the cell population were agglutinable (+ + + +). Cells infected with strain Queensland were not susceptible to agglutination by this dose of con A for up to 48 h after infection.

con A receptors[21,24,25]. These, and our new findings, indicate that the inherent topography of con A receptors on cells with a high susceptibility to con A agglutination does not differ from that of normal cells which are not agglutinated, and that receptors cluster only after exposure to con A at room temperature or above. Thus, it seems that a non-oncogenic virus such as NDV, malignant transformation by viruses and protease-treatment can all induce greater mobility of con A receptors within the plasma membrane. Whether or not a common mechanism is operating in each situation remains to be resolved.

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Effects of Cytochalasin B on Uptake and Release of Putative Transmitters by Synaptosomes and on Brain Actomyosin-like Protein

A LARGE variety of contractile cellular functions is thought to be associated with microfilamentous processes which can be disrupted by the fungal metabolite cytochalasin B (ref. 1). To this list of contractile processes have been added the stimulated release of secretory products[2-4] as well as transmitter agents[5]. Based in part on the isolation of actomyosin-like protein (neurostenin) from mammalian brain nerve ending preparations[6] a mechanism has been suggested for the exocytotic release of transmitter materials at synaptic endings involving contractile events similar to those occurring during muscle contraction[7]. We have examined the effect of cytochalasin B on the uptake by rat whole brain nerve ending preparations of noradrenaline, dopamine, glutamic acid and GABA and their K^+-stimulated release. We also studied the effect of cytochalasin B on the adenosine triphosphatase activity of the brain actomyosin-like protein and muscle actomyosin.

The assay system for the uptake and release studies was the same as that used previously[8]. The cytochalasin B (ICI) was prepared as a 0.1 M solution in dimethylsulphoxide (DMSO) and added to a final concentration of 0.15 mM. Control studies were run with the same amounts of DMSO. In one set of experiments the synaptosome preparation was incubated with cytochalasin B for 15 min before the addition of 7-^{3}H-L-noradrenaline, 1,2-^{3}H-dopamine, U-^{14}C-L-glutamic acid or 1-^{14}C-4-aminobutyric acid (GABA) followed in 15 min (5 min for glutamic acid) by the addition of 1 M KCl to a final concentration of 30 mM K^+ (Fig. 1). The concentration of cytochalasin B used in these experiments was several-fold greater than that used by some workers[4] but comparable to that used by others[5,9].

Cytochalasin B had a small but significant effect on the uptake of noradrenaline and dopamine (Fig. 1*a*). Its major effect was on the K^+-stimulated release of noradrenaline (Fig. 1*b*); that on dopamine was less but also significant. Neither the uptake nor the release of glutamic acid or GABA was affected (Fig. 1, Table 1). Although the uptake of noradrenaline was inhibited approximately 20%, its release was inhibited by approximately 60% (Table 1). The effect on dopamine was not as dramatic; its uptake and release were inhibited approximately 30% and 25%, respectively (Table 1). Since there was no effect on uptake of amino acids, cytochalasin B seems not to influence energy metabolism in these experiments[8].

In a second set of experiments, the synaptosome preparation was incubated with the radioactive tracer for 15 min before the addition of the cytochalasin B, followed in 15 min by KCl to a final concentration of 30 mM K^+.

The noradrenaline, dopamine and GABA continued to

Table 1 Comparative Effects of Cytochalasin B on Uptake and K⁺-stimulated Release of Putative Transmitters

	% Control uptake*	% Control*	Release†
^{3}H-Noradrenaline	81±5‡ (5)	38±6‡ (5)	11±4‡ (4)
^{3}H-Dopamine	69±2‡ (5)	78±6‡ (5)	68±5‡ (4)
^{14}C-GABA	101±4 (5)	98±3 (5)	91±8 (2)
^{14}C-Glutamate	103±13 (4)	93±1 (4)	—

* Derived from studies described in Fig. 1*a* and *b*.
† Derived from the experiments described in Fig. 1*c* and *d*.
‡ $P<0.01$.

See Fig. 1 for experimental details. Uptake is defined as the ratio of: (d.p.m. mg^{-1} protein on filter)/(d.p.m. total filtrate) and release is defined as the ratio of uptake after addition of 30 mM K⁺ to the uptake before that addition. The values are expressed as the percentage of uptake or release in the absence of cytochalasin B±s.e. (*n*) is number of different preparations. Duplicate determinations were run with each preparation.

concentrate slightly in the tissue preparation during the second 15 min period even in the presence of the cytochalasin B (compare Fig. 1*a* with 1*c*). Cytochalasin B seemed to have no significant effect on the uptake of the compounds during this second 15 min period. In contrast, there was again a marked inhibition of the K⁺-stimulated release of noradrenaline (89%) (Fig. 1*d*); the effect on dopamine release was much less (30%) although also significant. There was no effect on the uptake or release of GABA.

The release of dopamine was much less affected than that of noradrenaline. The explanation for this may be that synaptosomes prepared from whole brains are more enriched with noradrenaline-specific endings than with dopamine-specific endings. The finding that neither the uptake nor release of glutamic acid and GABA were affected by cytochalasin B suggests that these substances are taken up and stored differently than the catecholamines[8,10–12]. Noradrenaline and dopamine are presumably stored in the presynaptic vesicles of the nerve endings and released from these sites. If this is true, then the amino acids may not be similarly stored.

The actomyosin-like protein, neurostenin, was isolated from the synaptosomal fraction of rat brain[6]. The Ca^{2+} or Mg^{2+} stimulated adenosine triphosphatase activity was measured by the P$_i$ released from ATP[13]. Incubation of the protein with cytochalasin B for 10 min at 37° C before the addition of substrate ATP (MgATP) resulted in approximately 50% inhibition of the Mg^{2+}-stimulated adenosine triphosphatase activity with a concentration of 0.1 mM cytochalasin B (Fig. 2). Only slightly greater inhibition was achieved at 0.5 mM cytochalasin B. The Ca^{2+}-stimulated adenosine triphosphatase activity was affected only to a small extent even at 0.5 mM cytochalasin B. With concentrations at or below 0.1 mM the inhibition of the reciprocal of the rate of ATP hydrolysis was proportional to the concentration of the cytochalasin B (Fig. 2). These data suggest the inhibition is of a non-competitive nature.

Table 2 Effect of 0.1 mM Cytochalasin B on Mg^{2+}-stimulated Adenosine Triphosphatase Activity

Myosin + actin	Myosin + neurin	Stenin + actin	Stenin + neurin
% Control activity			
54±5 (2)	31±4 (4)	47, 15 (2)	56±4 (4)

See Fig. 2 for details of assay conditions. The protein concentrations in the incubation mixtures were 0.01 to 0.02 mg ml^{-1} for myosin and stenin and 0.02 to 0.05 mg ml^{-1} for actin or neurin (*n*)=number of preparations. Duplicate determinations were made on each preparation.

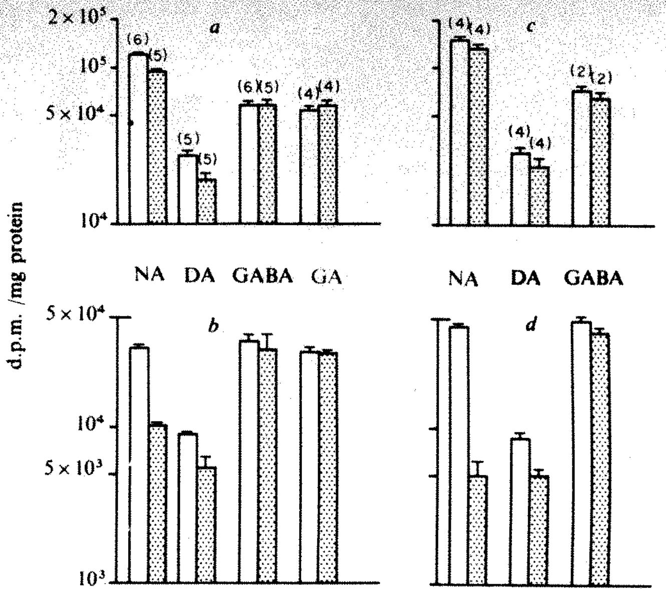

Fig. 1 Effect of cytochalasin B (CB) on the uptake by rat brain synaptosomes of noradrenaline (NA), dopamine (DA), 4-aminobutyric acid (GABA) and glutamic acid (GA) and their K⁺-stimulated release. □, Control; ■, 0.15 mM CB. *a*, *b*, Portions of crude synaptosomal preparations were incubated 15 min at 37° C with or without 0.15 mM cytochalasin B in a Krebs-Ringer phosphate buffer (*p*H 7.3–7.4) fortified with 10 mM glucose, 0.45 mM ATP and 10 mM pyruvate[8]. When NA or DA were used, the medium also contained 0.03% (w/v) ascorbate and 0.001% pargyline. When GABA or glutamate were used, 1 mM aminooxyacetic acid was added to the basic medium. The protein concentration was 1.0–1.5 mg ml^{-1}. At the end of the 15 min incubation period, L-7-^{3}H-noradrenaline (6.6 Ci mmol^{-1}), 1,2-^{3}H-dopamine (370 mCi mmol^{-1}), 1-^{14}C-4-aminobutyric acid (3 mCi mmol^{-1}) or L-U-^{14}C-glutamate (270 mCi mmol^{-1}) were added; the final concentrations were 0.20 μM, 0.25 μM, 20 μM and 0.2 μM, respectively. Fifteen minutes following addition of radioactivity (5 min for glutamate) 1 ml samples were removed from the incubation mixture and passed through a 0.65 μm Millipore filter. The filter was washed three times with 1 ml of 0.15 M NaCl. At the same time 1 M KCl was added to the incubation mixture to yield a final concentration of 30 mM K⁺. After 3 min, a 1 ml sample was removed for analysis and treated as above. Radioactivity on the filters and in the filtrates was measured with a Packard Tri-Carb liquid scintillation spectrometer. *a*, Radioactivity on the filter before addition of the KCl; *b*, radioactivity released into the medium by the addition of KCl. *c*, *d*, These experiments were similar to *a* and *b* except that the preparations were incubated with label for 15 min at 37° C and then 0.15 mM cytochalasin B added. The mixtures were then incubated for a further 15 min. At that time, a 1 ml sample was withdrawn for analysis and KCl added as before. *c*, Radioactivity on the filter before KCl addition; *d*, radioactivity released into the medium by addition of KCl. Since the cytochalasin B was added as a solution in dimethylsulphoxide, the controls received similar volumes of dimethylsulphoxide alone. (*n*)=number of separate preparations tested in duplicate. In *a*, *b* and *d*, the difference in d.p.m. mg^{-1} protein between control and addition of cytochalasin B for NA and DA was significant at the $P<0.01$ level by Student's *t* test.

The neurin and stenin were obtained after osmotic shock disruption of synaptosomal preparations[7]. The neurin was isolated either by direct extraction from the membrane fraction[7] or from the osmotic shock supernatant medium remaining after separation of the vesicle and membrane fractions. In the latter case, the supernatant was made 0.1 M in KCl, 1 mM in MgCl$_2$ and 0.5 mM in ATP. After 1 h at room temperature in the dark, the protein was allowed to polymerise overnight at 4° C. After centrifugation at 100,000*g* for 3 h the pellet was solubilised in 0.2 mM ascorbate, 0.2 mM ATP, *p*H 7.8 and the solution clarified by centrifugation for 20 min at 47,000*g*. The neurin prepared by the two procedures behaved similarly in the recombination studies described below and therefore the resulting data were pooled. The stenin was isolated from the osmotic shock vesicle fraction[7]. Actin and myosin were prepared from cat striated muscle[14,15].

In recombination studies (Table 2), 0.1 mM cytochalasin

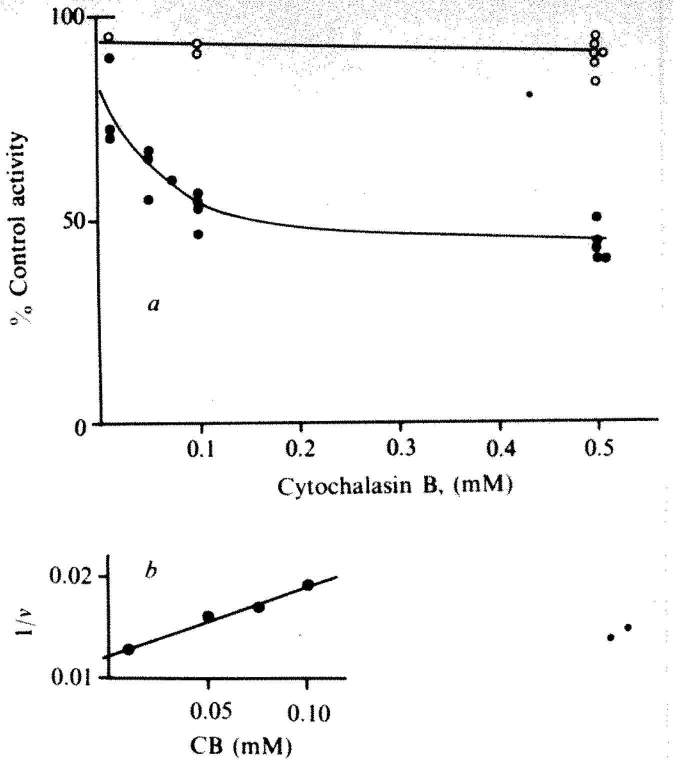

Fig. 2 *a*, Effect of cytochalasin B on the adenosine triphosphatase activity of actomyosin-like protein (neurostenin) isolated from rat brain synaptosomal preparations[6]. The enzyme activity was measured as µmol P_i released per mg protein per 30 min. Incubation medium contained 0.03 M imidazole, 0.06 M KCl, 0.5 mM ATP; 1 mM Mg^{2+} or Ca^{2+}, 0.1 mM ouabain and 0.1 mg protein, final volume 1 ml, *p*H 7.2. Samples were preincubated 10 min at 37° C without ATP, in the presence or absence of various concentrations of cytochalasin B. Controls contained equal amounts of dimethylsulphoxide. The data were expressed as the percentage of control activity. Each point is the average of duplicate determinations. ○, Ca^{2+}; ●, Mg^{2+}. *b*, Inverse of rate of enzyme activity against cytochalasin B concentration (CB).

B inhibited the Mg^{2+}-stimulated adenosine triphosphatase activity approximately 50%. This was equally true for myosin plus actin, myosin plus neurin, stenin plus actin and stenin plus neurin.

These studies clearly demonstrate that cytochalasin B inhibits the Mg^{2+}-stimulated adenosine triphosphatase activity of reconstituted muscle actomyosin as well as that of the analogous protein from nerve ending preparations. The effect on the Mg^{2+}-stimulated but not on the Ca^{2+}-stimulated enzyme activity suggests that the action of the drug is on the interaction between actin and myosin. It has been suggested that cytochalasin B disrupts actin-like microfilaments[1]. Although Wessels *et al.*[1] found no effect of cytochalasin B on the ability of striated muscle actomyosin to 'super-precipitate', Spudich and Lin[9] did find that this compound caused a decrease in the viscosity of the actomyosin complex, as well as of F-actin, and inhibited the adenosine triphosphatase activity of the actin-heavy meromyosin complex; they interpreted their findings as indicating that cytochalasin B interacted with actin. Smooth muscle contraction and cardiac muscle contraction have also been described as sensitive to cytochalasin B[1] as is the fibrillogenesis of developing muscle cells of chick embryos[16]. The drug also reversibly stops the beating of the developing chick heart[16].

We have proposed[7] that the process of exocytosis by which transmitter material is released at synaptic endings is in part attributable to a chemomechanical interaction between stenin associated with presynaptic storage vesicles and neurin associated with the presynaptic membrane or neurofilaments. The effects of cytochalasin B on the K^+-stimulated release of noradrenaline and on the Mg^{2+}-stimulated adenosine triphosphatase activity of brain actomyosin-like protein give further support to this proposal.

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Skin Temperature Modifies the Pleasantness of Thermal Stimuli

MUCH of human temperature regulation is mediated by behaviour, and thermoregulatory behaviour is guided in large measure by thermal sensations, particularly their affective components, that is, their pleasantness or unpleasantness. When we feel unpleasantly cold or warm, we turn the thermostat up or down, put a sweater on or take it off.

The thermal environment that results from regulatory activity is one that the person finds pleasant, or at least not unpleasant. What a chosen environment will be, that is, at what skin temperatures the person will feel comfortable, depends significantly on internal body temperature. The same temperature on a portion of the skin can seem pleasant or unpleasant given different internal temperatures[1]. It seems likely, however, that much behavioural thermoregulation takes place at constant internal temperatures[2,3]. It would be disadvantageous to wait until internal temperature rises or falls before a person takes appropriate action.

Given this line of reasoning, we predict that pleasantness and unpleasantness of thermal stimuli should depend on the temperature of the skin before stimulation—which itself reflects environmental conditions—given constant internal body temperatures. Yet some observations[1,4] suggest that pleasantness and unpleasantness depend primarily, if not solely, on internal temperature. Cabanac[1] found that ratings of affect produced by warming and cooling the hand to various temperatures was independent of mean skin temperature, which was controlled by immersing the entire body in water. In the study reported here, we have shown that affective responses do depend on superficial skin temperature, at least when it is varied by changing the temperature of ambient air, and a portion of the subject's skin is selectively stimulated by radiation. Thus our data agree with the familiar behavioural response of people who light fires in the winter, but not in the summer, to heat their hands and faces.

We applied the method of magnitude estimation[5] to scale the perceived affective intensity of transient (3-s) exposures of 21.8 cm^2 of the forehead to thermal radiation. Stimuli were presented in random order, at the rate of one every 45 s. Subjects stated whether each stimulus was pleasant or unpleasant, then assigned a number in proportion to the degree of pleasantness or unpleasantness. Subjects were instructed to assign numbers of equal magnitude to stimuli that produced equal degrees of pleasantness and unpleasantness. Each subject's forehead was blackened with India ink to promote absorption of the radiation, which was produced by a quartz lamp. A thermocouple monitored the temperature of the portion of the forehead that was stimulated, and, in two of the subjects, internal (oesophageal) temperature was measured continuously with a thermocouple-tipped catheter. Both thermocouples were copper-constantan junctions. The oesophageal thermocouple was inserted at the level of the heart: oesophageal temperature closely follows the temperature of the blood[6]. Five young men served as subjects. In all test sessions, they were dressed in light-weight clothes: however, in sessions run at low ambient temperatures (5° and 10° C), they wore a laboratory coat over their clothes.

Figure 1 shows, for two of the subjects, how mean magnitude estimates relate to both stimulus irradiance and rise of skin temperature; these results are typical of all five subjects. Local sensation on the forehead to the ambient air was close to neutral in affect when no heat was applied. As stimulus irradiance increased, the pleasantness of the sensation increased to a maximum, and then declined to neutrality, after which the sensation became more and more unpleasant. The exact shape of the curve depended strongly on ambient air temperature: when ambient temperature was low (5° and 10° C), pleasantness increased to relatively high values, and the sensation became unpleasant—though not painful—only at high stimulus intensities.

All test sessions lasted less than 40 min, sessions at the extreme temperatures (5° and 30° C) less than 30 min. The use of brief sessions made it unlikely that internal body temperature would have time to change; measurements taken with the oesophageal thermocouple on these two subjects confirmed the constancy of internal body temperature at 37° ± 0.2° C.

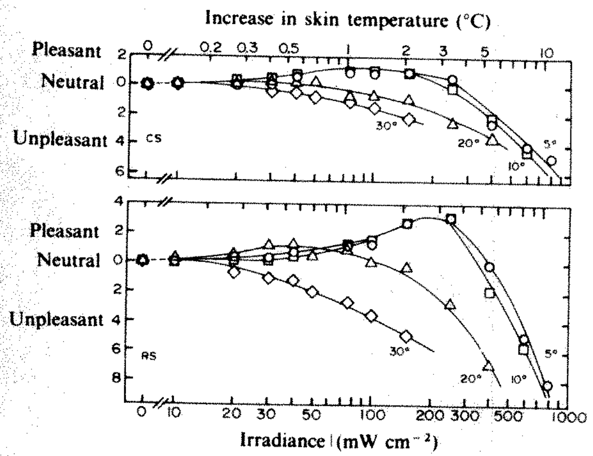

Fig. 1 Average magnitude estimates of pleasantness and unpleasantness as functions of both stimulus irradiance (lower abscissae) and resulting increase in skin temperature (upper abscissae). The parameter is air temperature. Each point is the average of two or three judgments per session made during two or three sessions.

Clearly, the affective responses of the thermal system are determined in part by signals arising from the skin. The lower the ambient temperature, the lower the initial skin temperature and the greater the rises in skin temperature that are perceived as pleasant. Cabanac[1] found that the affective responses varied with both temperature of the stimulated skin and internal body temperature, but not at all with mean skin temperature. In the experiment described here affective responses did depend on the temperature of the skin before stimulation. A decrease in the ambient temperature from 20° to 5° C resulted in a decrease of forehead temperature by only 1° C for subject C. S. and 2° C for subject R. S. But the curves in Fig. 1 obtained at 5° C air temperature are displaced more than 1° and 2° from curves obtained at 20° C. Our results demonstrate, therefore, that affective responses vary with both pre-stimulus temperature of the skin and the temperature of the skin produced by radiation. An increase of skin temperature to 34° C, for example, was felt as very pleasant in cold air, but unpleasant in warmer air. The difference between our results and those of Cabanac[1] may be due to use of radiant rather than conducted heat or to different affective responsivity of the forehead and hand.

Even in constant environmental conditions, the affective dimension of thermal sensation is not determined solely by the rise in temperature produced by heat. We also found that pleasantness and unpleasantness display spatial summation. An example of results is shown in Fig. 2. When areas of 3.68 and 21.8 cm^2 of the forehead were stimulated alternately in the same sessions, it became clear that size of the area that is stimulated counts as importantly as how much heat is applied. Warmth sensation itself displays generous spatial summation, in that over low and moderate stimulus irradiances, doubling the stimulated area is about as effective as doubling the irradiance[7,8]. As Fig. 2 shows, spatial summation is also a prominent feature of thermal affect.

We conclude therefore that the affective dimension of thermal sensations produced by transient stimulation is determined at least in part by the thermal signals integrated over the skin. Our results are consistent with an extension of Cabanac's hypothesis[1,9] that affect tends to improve or restore homeostasis. The ambient temperatures 5° and 10° C that we used would, under long exposure, lower internal temperature. The affective responses to transient heating seem, then, to be anticipatory to the extent that they are homeostatic; Gagge *et al.*[10] noted this anticipatory component to transient thermal stimuli. Heat in the cold is pleasant, and, to some extent, enough of it helps to keep internal body temperature from

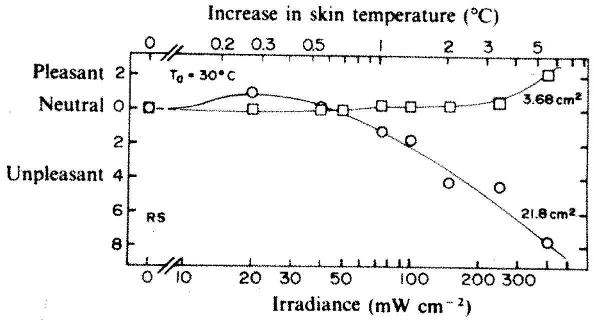

Fig. 2 Average magnitude estimates of pleasantness and unpleasantness as functions of both stimulus irradiance (lower abscissa) and resulting increase in skin temperature (upper abscissa). The results depend strongly on the extent of the area stimulated. Each point is the average of two judgments per session made during three sessions.

falling. But thermal homeostasis may not be the whole story. High-irradiance stimulation is perceived in cold air as unpleasant, even though it does not suffice to make up for heat that has been lost. This perception of unpleasantness may serve to warn the organism of possible pain, and tissue destruction that will result if the heat stimulus continues.

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Release of Lysosomal Enzymes by Alveolar Mononuclear Cells

THE finding that individuals severely deficient in the circulating enzyme inhibitor α_1-antitrypsin usually contract panlobular emphysema at an early age suggested that proteolytic enzymes were involved in the connective tissue destruction characteristic of pulmonary emphysema[1]. This idea was strengthened by reports that intratracheal administration of the relatively nonspecific enzyme, papain, or the more specific enzymes, elastase or collagenase, led to the development of emphysema-like lesions in a variety of animal species[2,3]. The origin of such proteolytic enzymes *in vivo*, and their mode of release has been the subject of much speculation, and two cell types, the polymorphonuclear (PMN) leukocyte and the alveolar macrophage are implicated. Evidence favouring the PMNs include their sequestration in the basilar portions of the lung in the non-diseased person, their containing large numbers of lysosomes, and the high incidence of concurrent infection (with resultant leukocyte infiltration) associated with non α_1-antitrypsin related emphysema. In addition human PMNs release approximately 25% of their lysosomal enzymes during phagocytosis of various particles such as zymosan, latex, and immune complexes[4].

Evidence also exists favouring the involvement of alveolar macrophages in the pathogenesis of emphysema: these cells contain larger amounts of certain lysosomal enzymes[5], have an elastase which is not inhibited by α_1-antitrypsin[6], and are present in the lung in high concentrations during infectious and non-infectious states. Rats exposed to low levels of NO_2 develop emphysema-like lesions without infiltration of polymorphonuclear leukocytes[7]. Peritoneal macrophages have recently been shown to release approximately 15% of their enzyme content during phagocytosis[4]. To determine if the alveolar cells likewise release a portion of their lysosomal enzymes during phagocytosis mononuclear cells lavaged from the guinea pig lung were incubated with zymosan particles, and the concentrations of β glucuronidase and elastase, lysosomal enzymes, and lactate dehydrogenase (LDH), a cytoplasmic enzyme which leaks from dying and dead cells, were monitored in the suspending media and cells. Selective release of lysosomal enzymes occurs when proteolytic enzymes, but not LDH, are found in the media.

Male Hartley strain guinea pigs, 250–300 g were injected intracardially with 0.2 ml complete Freund's adjuvant (Fisher) to stimulate production of alveolar mononuclear cells[8]. Two weeks later the animals were anaesthetised with sodium pentobarbital, 40 mg kg^{-1}, and exsanguinated from the jugular vein. Blood was allowed to clot for 2 h at room temperature and the serum collected. The thoracic cavity was opened and the pulmonary artery perfused with saline until the pulmonary circulation was free of blood. The heart and lungs were removed, blood washed from their surfaces, and the lungs lavaged with saline until 110 ml was collected. After collection the cells were counted, centrifuged, and resuspended in Medium 199 (Microbiological Associates) at a concentration of 10^6 cells ml^{-1}.

During the cell collection and preparative manipulations, zymosan particles (Nutritional Biochemical Company) were

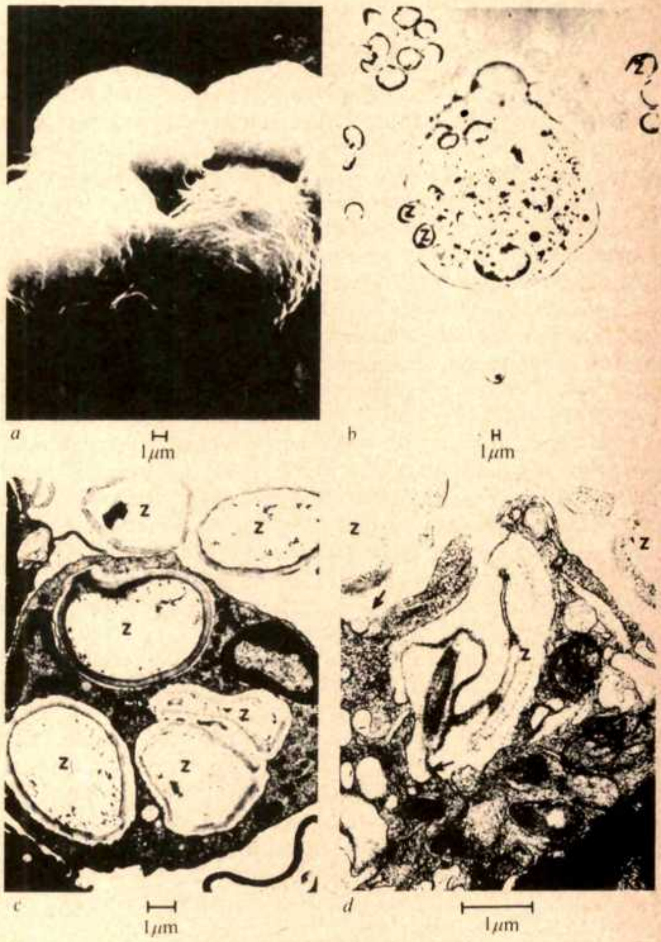

Fig. 1 *a*, Scanning electron micrograph of guinea pig alveolar mononuclear cells. Cells were fixed in 2.5% glutaraldehyde, washed in distilled water, air dried, coated with gold-palladium to a depth of 200 Å, and examined with a Jeol Scanning Electron microscope at an accelerating voltage of 25 kV. Note differences in the sizes of cells and the degree of roughness of the external membranes. *b*, Alveolar mononuclear cell phagocytosing zymosan particles (Z) as seen with phase contrast microscopy. A large amount of granular material is scattered throughout the cell. *c*, Transmission electron micrograph of alveolar cells incubated with zymosan (Z). Cells were fixed with buffered glutaraldehyde, 2.5%, post-fixed in osmium tetroxide, stained with uranyl acetate in 50% ethanol, embedded in epoxy resin, and examined with a Siemens Electron Microscope. Electron dense material is present within the phagolysosomes (arrows). *d*, Transmission electron micrograph of alveolar cells and zymosan. A phagocytic vacuole apparently open at the cell periphery with electron dense material being extruded into its internal border ('regurgitation during feeding'). At the interface between a zymosan particle (Z) and the cell a lysosome is releasing its contents directly to the outside (reversed endocytosis).

Table 1 Effects of serum and gaseous environment on β glucuronidase and LDH release from alveolar mononuclear cells

Conditions	*n*†	β Glucuronidase: Incubation without particles (mean ± s.d.)	β Glucuronidase: Incubation with particles (mean ± s.d.)	*P*‡	Lactate dehydrogenase: Incubation without particles (mean ± s.d.)	Lactate dehydrogenase: Incubation with particles (mean ± s.d.)	*P*	Percentage uptake§ (mean ± s.d.)
		Enzyme release*						
Cells + isologous serum in 95% O_2–5% CO_2	6	8.8 ± 2.0	26.6 ± 2.7	<0.01	7.0 ± 1.3	9.5 ± 0.6	<0.2	89.3 ± 1.4
Cells + isologous serum in air	6	11.8 ± 2.3	17.3 ± 2.3	<0.2	7.3 ± 1.0	10.2 ± 1.9	<0.2	86.2 ± 2.9
Cells + autologous serum in 95% O_2–5% CO_2	6	8.3 ± 2.0	26.3 ± 2.4	<0.01	7.0 ± 2.0	7.7 ± 1.3	<0.8	92.6 ± 0.4

* β Glucuronidase and lactate dehydrogenase values are the percentage of total enzyme released into the supernatant during a 2 h incubation period under the specified conditions. Cell levels were 1.49 ± 0.59 nM β glucuronidase per million cells per min and 141 ± 43 absorbancy units LDH per million cells (mean ± s.d., twelve experiments each).

† Number of experiments.

‡ Probability as determined by Student's *t* test.

§ Percentage of cells containing two or more zymosan particles.

incubated for 30 min with fresh serum previously obtained. Ten million cells in Medium 199, a 0.5% solution of zymosan, and 10% fresh guinea pig serum (final volume 2 ml) were incubated for 2 h in a Dubnoff shaker, 37° C, and gassed with either air or 95% O_2–5% CO_2. The mixtures were immediately placed on ice, centrifuged at 500*g* for 10 min and the supernatants assayed for β glucuronidase, elastase, and LDH. One aliquot of cells was resuspended in acetate buffer, 0.1 M, *p*H 4.5, containing 0.1% Triton X-100 (Sigma) and sonicated with an E/MC Ultrasonic cleaner oscillating at 90 Hz s^{-1} and assayed for β glucuronidase activity using a phenolphthalein glucuronide (Nutritional Biochemical Company) substrate[9]. Another aliquot was suspended in the same buffer without Triton X-100, sonicated for 10 min, and assayed for elastase using t boc-l-alanine nitrophenyl ester (Cyclo) substrate in phosphate buffer, 0.05 M, *p*H 6.5 (ref. 10). The elastase activity determined after sonication without Triton X-100 was equivalent to that found after ten freeze-thawing cycles indicating that the lysosomes had been cleaved by the sonication procedure. LDH levels were determined by measuring the initial rate of oxidation of $NADH_2$ (Sigma) in the presence of 0.01 M sodium pyruvate[11].

The degree of phagocytosis was ascertained by counting the number of cells which had engulfed at least two zymosan particles. A minimum of 100 cells was examined.

The cells were viewed under phase, transmission, and scanning electron microscopy. Over 80% were monocytes or macrophages, approximately 15% were lymphocytes, and the remainder were eosinophils. Few if any basophils, neutrophils, or erythrocytes were found in the preparations. When viewed under scanning electron microscopy virtually all cells had the ruffled membranes typical of phagocytic cells (Fig. 1*a*).

Table 2 Release of elastase from alveolar mononuclear cells exposed to zymosan particles*

	n†	Elastase activity (ΔA per 30 s): Incubation without particles (mean ± s.d.)	Elastase activity (ΔA per 30 s): Incubation with particles (mean ± s.d.)	*P*‡
Cells	4	0.0143 ± 0.0022	0.0060 ± 0.0011	<0.01
Supernatant	4	0.0175 ± 0.0064	0.0160 ± 0.0061	n.s.
Serum	4	0.0187 ± 0.0069	0.0170 ± 0.0050	n.s.

* Ten million alveolar mononuclear cells, 0.5% zymosan, and 10% fresh isologous serum (final volume, 2 ml) were incubated for 2 h with 95% O_2–5% CO_2 gas mixture. The cells were centrifuged, resuspended in 2 ml 0.05 M sodium acetate buffer, *p*H 4.5, and sonicated. 0.2 ml of the supernatant solution and 0.2 ml of the sonicate were assayed for elastase activity with t boc-l-alanine nitrophenyl ester as substrate.

† Number of experiments.

‡ Probability as determined by Students *t* test.

Approximately 90% of the cells phagocytosed the zymosan particles, and the uptake was independent of the gaseous environment or serum sources employed (Table 1; Fig. 1*b* and *c*). Those cells incubated with the zymosan, in either isologous or autologous sera, and in the presence of 95% O_2–5% CO_2, released more than three times as much β glucuronidase as the cells similarly treated but not exposed to the particles. Cells incubated with the particles in air released only slightly more β glucuronidase than the controls. Alveolar cells are continually exposed to a highly oxygenated environment *in vivo*, use more oxygen than either peritoneal macrophages or PMNs[12], and probably depend on an oxidative energy metabolism for phagocytic function and its sequelae, enzyme release.

Three mechanisms have been proposed to account for enzyme release from phagocytic cells. The first, initially described by Metchnikoff, involves the release of material from dead or dying cells. Release in this instance is 'non-selective', that is, cytoplasmic and lysosomal contents would be expelled concomitantly. Approximately 7% of the total LDH was found in the supernatants from cells not exposed to the zymosan; this percentage was enhanced by the presence of zymosan, especially when the environment contained air, but in no cases were the differences in LDH release between cells incubated in the presence and absence of zymosan statistically significant. The release, then, was selective in that the lysosomal but not the cytoplasmic enzyme marker was extruded from cells exposed to zymosan.

The second mechanism, termed reversed endocytosis, occurs when the lysosome migrates to the surface of the cell and expels its contents directly to the outside[13]. Such a pattern is normally observed when cells are exposed to non-phagocytosable surfaces[14], or to a phagocytosable substance in the presence of cytochalasin B[15]. The third mechanism, 'regurgitation during feeding', is characterised by the release of enzyme through the open end of an incompletely fused phagolysosome[16]. In Fig. 1*d* a cell demonstrating both reversed endocytosis and regurgitation during feeding is presented.

A second lysosomal enzyme, elastase, may be especially important in the pathogenesis of emphysema. Elastin isolated from emphysematous human lungs had significantly decreased ratios of non-polar/polar amino acids suggesting that the outer core of elastin, which is thought to be composed of the non-polar amino acids, was degraded by locally released elastase. The alveolar walls of rats exposed to the proteolytic enzyme thermolysin showed fragmentation, thickening, and degeneration characteristic of emphysema as well as a decrease in the percentage of non-polar amino acids[17]. In our experiments the elastase found in the supernatant from cells incubated with or without zymosan could be accounted for by the enzyme present in the serum; those cells incubated with zymosan, however, had significantly decreased levels of elastase (Table 2) indicating that the enzyme was released but was either rapidly inactivated[18] or adhered to the cell membrane[14].

The results of these experiments provide evidence that alveolar mononuclear cells can release proteolytic enzymes on phagocytic stimulation. These cells normally engulf particulate matter that passes the 'mucociliary escalator' which lines the upper respiratory passages and may release a portion of their enzyme load directly on the bronchiolar and alveolar surfaces. Under normal conditions the α_1-antitrypsin found in the lungs would be expected to inactivate the enzymes. In its absence, such as in the homozygous α_1-antitrypsin deficiency, or when the amount of particulate matter is excessive, sufficiently high levels of proteolytic enzymes may be free to cleave the pulmonary connective tissue.

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Immunisation with Antigen coupled to an Immunosorbent

IN attempts to produce antisera to surface components of cells one is often frustrated by the fact that many components are poorly immunogenic *in situ*, presumably due to antigenic competition from myriad adjacent structures. The alternative approach of preparing a component in pure form in an amount sufficient for immunisation schedules can be formidable. Advantage may be taken, however, of an intermediate stage in some schedules for purifying such components: specific adsorption onto a solid phase. For example, the receptors for con A and phytohaemagglutinin have been isolated in this way from detergent lysates of lymphocytic plasma membranes[1,2]; and the Fab fragments of surface immunoglobulins can be isolated on an immunosorbent from a proteolytic digest of the lymphocytic surface[3]. The availability of immunosorbents of high quality[4] has extended the scope of such methods.

I have investigated the feasibility of evoking an antibody response by direct injection of immunosorbent with its attached antigen. The aims are to minimise losses of the antigen by stopping the preparation at the stage of immunoadsorption, and to present what little antigen there is in an optimally immunogenic form. The principle of injecting antigen adsorbed to a specific solid phase is not new[5], but it has not been extended to the very flexible immunosorbents nor used with the small amounts of antigen often available, for example, from mammalian cell surfaces. The major apparent limitation is that to deal with an antigen having determinants ABC, an antiserum reacting with one or more of the determinants is needed initially (that is an antiserum to a cross-reacting antigen) to prepare the immunosorbent.

As a model I have chosen a requirement to raise antibodies to the V region[6,7] of the Fabγ fragment from a human myeloma protein, given first, a solution containing the Fabγ fragment at 1 μg ml^{-1} plus a contaminating protein, bovine serum albumin (BSA), at 1 mg ml^{-1}, and second, a rabbit antiserum to normal Fabγ, which by its nature will react with determinants on the C region but not the V region of the myeloma Fabγ.

The solid phase chosen was microcrystalline cellulose (catalogue number 2330; E. Merck, Darmstadt). This has a quoted particle size of 3 to 30 μm, but in fact a suspension in 0.1 M NaCl, 0.02 M Tris-HCl, *p*H 8.0, was seen to have many particles also between 1 and 3 μm. At several of the handling stages, particularly after activation with cyanogen bromide, it was seen to be aggregated, but this was reversible. It was chosen in preference to solids more commonly used for immunosorbents (Sepharose, Sephadex, coarser celluloses) because it has many particles of a size permitting phagocytosis. Whether this potential advantage is important in practice has not yet been tested.

Immunosorbent was prepared by coupling IgG (50 mg), from the rabbit anti Fabγ serum, to microcrystalline cellulose (1 g) at *p*H 6.5, using the cyanogen bromide method[8,9]. About 60% of the protein was coupled. In terms of packed volume of cellulose per volume of suspension, the most convenient measure of concentration, this yielded 55 ml of 5% immunosorbent. A blank immunosorbent was prepared by substituting IgG from non-immune serum in the above procedure.

In a typical experiment, 1 ml of 5% anti-Fabγ immunosorbent was added to 10 ml of the Fabγ–BSA solution and stirred constantly for 24 h at 4° C. 65% of the Fabγ was bound. Four washes with 0.1 M NaCl, 0.02 M Tris-HCl, pH 8.0, were carried out by displacement filtration in tubes previously described[10]. Previous adsorption with blank immunosorbent made no difference: as judged by immunofluorescence the blank had bound neither Fabγ nor BSA. (It should be emphasised, however, that in many situations it is vital to carry out blank immunoadsorption first to remove material adhering non-specifically to the matrix. This is especially important when dealing with cell lysates.) The test immunosorbent, when examined by immunofluorescence, was strongly positive for Fabγ but negative for BSA.

Rabbits were immunised with 0.01 ml packed immunosorbent bearing approximately 1 μg bound Fabγ. This was divided into equal primary and secondary doses given 5 weeks apart. Each dose was in turn divided into four subcutaneous injections, one into each shank. Table 1 compares the results obtained using various adjuvants. Freund's complete adjuvant (FCA) clearly gave the best antibody titres. No precipitins to BSA were detected in any of the sera. All subsequent experiments used FCA.

Injection into the footpads was not obviously more effective and it caused considerable inflammation. It was better to divide 1 μg of antigen into two doses given 5 weeks apart than to give it all as a single dose: this in spite of the fact that the antigen depots might be expected to yield antigen for some weeks. The antibody titre was maximal at about 2 weeks after the second dose.

The antibody response seemed to be divided approximately equally between the C and V regions of the Fabγ. It had been hoped that the V regions, projecting outwards, would provoke the stronger response. Previous sensitisation to the C regions, by subcutaneous injection of 0.1 mg normal Fabγ in FCA 1 week before the primary injection, diminished the response to the V regions. An attempt to make the animals tolerant to the C regions by giving 50 mg monodisperse IgG intravenously at the time of the primary injection failed. However passive antibody[11,12] to the C regions (60 mg IgG from the rabbit antiserum to normal Fabγ, given intraperitoneally on the day following the primary injection) markedly diminished the response to the C regions and probably enhanced that to the V.

Table 1 Antibody responses to bound Fabγ

Adjuvant and dose*	Antibody titre 2 weeks after secondary injection†
Nil	0 to 2
Freund's complete adjuvant (Difco), 50% of injected volume	4 to 16
Al (OH)$_3$ (Alhydrogel), 200 μg of Al	2 to 4
Polyacrylate (Primafloc AlO), 200 μg	1 to 4
B. pertussis, 2 × 10^9 organisms	1 to 2

* The total dose of Fabγ was 1 μg, bound to 0.01 ml packed immunosorbent. This was mixed with saline and adjuvant to give a volume sufficient for primary and secondary lots of four subcutaneous injections, each 0.3 ml. The dose of adjuvant shown is the total for the course, divided equally among all the injections.

† Range of titres observed in three or four rabbits. Given as the reciprocal of the greatest of doubling dilutions giving a precipitin line on an Ouchterlony plate with 5 μg antigen; the reagent wells were of 6 mm diameter with their centres 10 mm apart. A strong precipitating antiserum can give a titre of 256 by this method.

It is clear that the procedure described is capable of giving moderate antibody responses to minute amounts of bound antigen. I am continuing to evaluate the variables involved.

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Dual Action of Praseodymium (Pr^{3+}) on Transmitter Release at the Frog Neuromuscular Synapse

THE release of transmitter after a nerve action potential seems to require the entry of calcium ions through the presynaptic membrane[1,2]. Mitochondrial uptake within the nerve terminal constitutes a possible way of removing these calcium ions temporarily[3,4]. While inhibition of the first process, namely the entry of calcium, would be expected to decrease the mean number of quanta liberated by a nerve impulse (quantal content, *m*), selective inhibition of the second process would tend to augment the release of transmitter.

A substance that acts on both calcium transport processes, may have either an inhibitory or facilitatory effect, depending on the relative sensitivities of these two processes and the appropriate concentrations. Lanthanides are known to be strong inhibitors of calcium uptake by the mitochondria[5], and lanthanum (La^{3+}) is known to prevent entry of calcium ions into nerve[6,7]. We have attempted to distinguish between these two opposing actions using the lanthanide praseodymium (Pr^{3+}).

We used superficial fibres of the cutaneous pectoris nerve muscle preparation of the frog (*Rana pipiens*). Intracellular electrodes (3 M KCl, 40–90 MΩ resistance) were inserted into the muscle, usually employing Nomarski interference optics. The penetrations were made 200–300 μm from the nerve terminal. The preparations were kept in Ringer solution of the following composition: 116 mM NaCl, 2.0 mM KCl, 1.8 mM CaCl$_2$, 0.25 mM NaH$_2$PO$_4$. To block transmission and thereby prevent contractions, 10–12 mM MgCl$_2$ was added to the medium by isotonic substitution for sodium. Spontaneous activity (miniature end-plate potentials, m.e.p.p.) and evoked potentials (end-plate potentials, e.p.p.) were recorded conventionally.

Addition of 200 μM Pr^{3+} or more to the perfusion medium caused a rapid abolition of the e.p.p. (Fig. 1*a*). This effect of Pr^{3+} corresponds to that described for La^{3+} at the neuromuscular junction[8,9]. Another similarity of the action of these two lanthanides is the increase in spontaneous release: even at low concentrations, praseodymium causes a marked increase in the frequency of the spontaneous m.e.p.p.s (Fig. 2 *b*, *d* and *e*.).

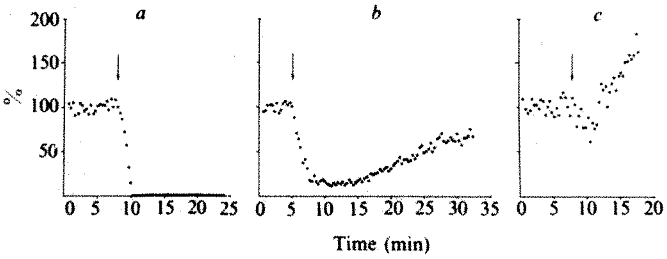

Fig. 1 The action of different Pr^{3+} concentrations on e.p.p. amplitude. Each point is the average value of 10 e.p.p's elicited at a rate of 0.5 s^{-1}. Bathing medium contained standard Ringer with 10 mM MgCl$_2$ to prevent contractions. Each experiment was performed on a different muscle. All values were normalised to mean initial control value of the end plate potential, R (given in parentheses below). *a*, The action of 250 μM PrCl$_3$ (control mean value $\bar{R}$=5.54 mV, *m*=19.07); *b*, 100 μM PrCl$_3$ ($\bar{R}$=5.86 mV, *m*=23.63); *c*, 15 μM PrCl$_3$ ($\bar{R}$=1.84 mV, *m*=5.36). All experiments were done at room temperature (20°–23° C).

However, at concentrations of 100 μM or less, the effect of

Pr^{3+} on e.p.p. amplitudes was not monotonically inhibitory: there was an initial decrease followed by an increase (Fig. 1*b* and *c*). Between 10 and 30 μM (Fig. 1*c*) the amplitude of the e.p.p. regularly increased beyond the initial control value during this late phase. An analysis of these responses reveals that the principal effect of Pr^{3+} is to reduce—and subsequently increase—transmitter output. Figure 2 shows that the early and late effects on e.p.p. amplitudes are reflected in corresponding changes in *m*, the number of quanta liberated by the nerve impulse.

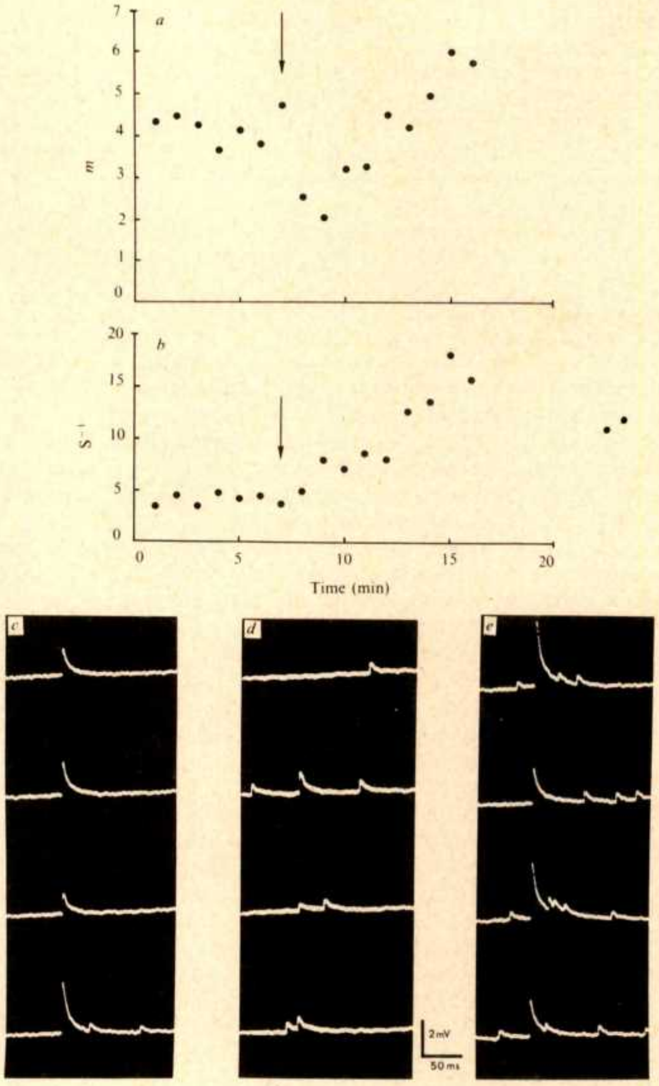

Fig. 2 Biphasic action of 15 μM $PrCl_3$ on evoked transmitter release. *a*, Effect on quantal content (*m*). Note the "dip" in *m*. Each point represents the mean value of twenty responses. No failure of transmission was observed in 140 responses elicited before addition of Pr^{3+}. *b*, Effect on m.e.p.p. frequency. Note that the effect on spontaneous release does not show a "dip". *c*, Sample records before the addition of $PrCl_3$. *d*, Sample records 3 min after addition of 15 μM $PrCl_3$. Note failure of transmission in upper trace. *e*, Sample records 8 min after $PrCl_3$. Stimulation frequency 0.33 s^{-1}. All records are taken from the same fibre. Other experimental details as in Fig. 1.

We interpret these results as follows. Addition of Pr^{3+} causes a decrease in the Ca^{2+} influx induced by the action potential. This will account for the abolition of the e.p.p. at high Pr^{3+} concentrations and for the moderate initial decrease (early phase) at low concentrations. La^{3+} does not interfere with action potential invasion of the nerve terminal[8], and it seems unlikely that Pr^{3+} acts differently in this respect (at much lower concentrations). If Pr^{3+} penetrates the nerve terminal (and there are indications that even larger molecules are able to do so[10–12]) it will interfere with calcium sequestration by the mitochondria (and perhaps also with other calcium removal processes). Hence $[Ca^{2+}]$ will increase in the cytoplasm of the terminal, causing the observed increase in m.e.p.p. frequency[13,14]. The evoked Ca^{2+} influx will sum with this increased internal concentration, to produce a higher quantal liberation. This secondary effect will only be observed at low levels of Pr^{3+}, when the Ca^{2+} entry sites are far from saturated by the antagonist, and significant amounts of Ca^{2+} ions still enter the nerve terminal after each action potential. Alternatively the observed increase in *m* may be attributed to changes in membranal ionic conductances. La^{3+} is reported to prolong the voltage dependent K^+ conductance in nerve cells[15,16], and would thus prolong the action potential. This effect, however, is seen at La^{3+} concentrations approximately three orders of magnitude higher than the Pr^{3+} concentrations used here. Thus we tend to favour the former hypothesis.

In conclusion, the observed effects of Pr^{3+} on evoked and spontaneous release may suggest active mitochondrial regulation of internal $[Ca^{2+}]$, and similarities between mitochondrial and membranal Ca^{2+} transport systems[4]. We also suggest that the increase in m.e.p.p. frequency caused by La^{3+} (ref. 8) and Pb^{2+} (ref. 17) is, at least partially, due to interference with mitochondrial Ca^{2+} sequestration.

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Does α-Bungarotoxin Inhibit Motor Endplate Acetylcholinesterase?

α-BUNGAROTOXIN, a specific and irreversible binding agent of acetylcholine receptors of the motor endplate[1,2] and of other nicotinic cholinergic receptors[3], has no inhibitory effect upon acetylcholinesterase preparation from electroplax of electric eel[3]. Štalc and Župančič[4] have, however, reported that, when a minced preparation of mouse diaphragm was used, α-bungarotoxin as well as *d*-tubocurarine markedly inhibited the membrane acetylcholinesterase when acetylcholine concentrations lower than 12 μM were used. They took this result as evidence to support their hypothesis that the anionic centre of the enzyme in the motor endplate is the acetylcholine receptor[5]. We attempted, therefore, to re-examine the effect of α-bungarotoxin by using intact acetylcholinesterase in the motor endplate in order to test the hypothesis and to establish the specificity of binding of the toxin.

α-Bungarotoxin isolated by fractionation on column chromatography[6,7] was found to be slightly contaminated with acetylcholinesterase existing in the crude venom of *Bungarus multicinctus*. The toxin solution, 1 mg ml^{-1} in acetate buffer, *p*H 4.0, was heated to 70° C for 20 min to completely decompose the enzyme activity. That α-bungarotoxin was not affected by this treatment was confirmed by its neuromuscular blocking action. The enzyme activity was measured on the intact isolated skeletal muscles according to the method proposed by Mittag *et al.*[8]. This procedure measures only the membrane enzyme located at the endplate. Two hemidiaphragms from each of several Long Evans strain rats of either sex weighing about 250 g, or four hemidiaphragms from each of several NIH strain adult mice, or six biventer cervicis muscles from each of several 7 to 15 d old male Leghorn chicks, were quickly isolated and immersed in an organ bath containing 10 ml Tyrode solution at 37° C and oxygenated with 95% O_2 and 5% CO_2. After 30 min incubation, 1 μM ^{3}H-acetylcholine (290 mCi mmol^{-1}, Radiochemical Centre, Amersham) was added and the enzymatic hydrolysis measured for a 10 min period of incubation. About 20% of the added acetylcholine was hydrolysed during this period. Aliquots (1 ml) of bath medium were passed through a column (6×25 mm) of Dowex 50 to remove unhydrolysed acetylcholine, and counted on a liquid scintillation counter. The enzyme activity was expressed as the rate constant according to the following formula[8]:

$$\text{Rate constant (ml per min per muscle)} = [(R-S)V]/[N(T-A)]$$

where R is the total increase of 'acetate' c.p.m. ml^{-1} min^{-1}; S, the spontaneous hydrolysis ml^{-1} min^{-1} in c.p.m.; V, the bath volume in ml; N, the number of muscles; T, the total c.p.m. per ml of the bath media; and A, the 'acetate' c.p.m. at zero time ml^{-1}. After the first 10 min measurement period, the test muscle preparation was incubated with α-bungarotoxin at 1 μg ml^{-1} for 70 min to completely block the neuromuscular transmission and to bind most of the acetylcholine receptors[9]. Then the enzyme activity was measured again in the presence of the toxin and expressed as a percentage of the first measurement. The preparation was then washed several times with plain Tyrode solution and, after 60 min, the enzyme activity was again measured. The results summarised in Table 1 illustrate that the enzyme of rat diaphragm was quite stable over the experimental period of about 4 h. It was found that when most of acetylcholine receptors were bound with α-bungarotoxin, the enzyme activity was slightly increased instead of markedly inhibited as reported by Štalc and Župančič. Similar results were obtained for the mouse diaphragm and chick biventer cervicis muscle. The enhancement of enzyme activity by the toxin seemed to be irreversible. No defect except a slight enhancement was observed even at ten times higher concentrations of α-bungarotoxin. *d*-Tubocurarine at a concentration of 1 μg ml^{-1}, which significantly blocked the neuromuscular transmission, also showed no inhibitory effect on the enzyme. About 20% inhibition was obtained at ten times higher concentration (Table 1). The enzyme, however, was completely inactivated by 0.01 μg ml^{-1} echothiophate, a specific inhibitor of acetylcholinesterase.

Table 1 Effect of α-bungarotoxin on endplate membrane acetylcholinesterase of rat diaphragm

Agents	*n*	Cholinesterase activity: Before treatment (1) (rate constant; ml per min per hemidiaphragm) ±s.e.	After treatment (2) (%±s.e.)	After washout (3) (%±s.e.)
Control	4	0.110±0.007	101±3.1	97±6.4
α-Bungarotoxin, 1 μg ml^{-1}	5	0.102±0.005	117±5.5*	117±7.6
α-Bungarotoxin, 10 μg ml^{-1}	4	0.123±0.005	114±3.8*	—
d-Tubocurarine, 10 μg ml^{-1}	3	0.104±0.105	81±4.8*	96±0.5

* $P < 0.05$ against control.

Control hydrolysis was measured for each hemidiaphragm before treatment. The second assay was performed in the presence of agents after 70 min incubation while the third one was after subsequent washings for 60 min. The activity was expressed as a percentage of the first assay.

Our results with α-bungarotoxin and *d*-tubocurarine are diametrically opposed to those of Štalc and Župančič. With their *p*H-static titration method, the sensitivity at a substrate concentration as low as 12 μM could be unreliable. Our results, therefore, indicate that the acetylcholine receptor is not identical with the anionic site of acetylcholinesterase. Barnard *et al.*[10] have shown that binding of α-bungarotoxin to the mouse diaphragm receptor did not affect the binding of diiso-propylfluorophosphate. These two components of electric organs of electric eel[11] and torpedo[12] have been separated. The slight degree of enhancement after neuromuscular blockade by α-bungarotoxin is interesting, however. Since the enhancement was the same in extent with either 1 or 10 μg ml^{-1} of the toxin and was irreversible, it is likely to be related to the irreversible occupation of the receptor. It may be that the blockade of the association of acetylcholine with the receptor increases the opportunity of the acetylcholine molecules to get access to the enzyme.

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The Mechanism of Auditory Evoked EEG Responses

ANALYSIS of the electroencephalogram (EEG) recorded over 0.5–1.0 s immediately following an auditory stimulus, is a well known objective method for evaluating auditory perceptual threshold, for example, in very young subjects. The low-level and visually indistinct individual responses occur in a spontaneous EEG background; hence repeated stimuli are used and the resulting ensemble of post-stimulus EEG signals averaged at each instant of post-stimulus time (PST). An exactly similar method is widely used in studying EEG visual evoked responses and other neuroelectric phenomena[1]. This averaging procedure is effective in clarifying any consistent response in the presence of spontaneous activity if identical responses are evoked by all stimuli and if the response waveform is uncorrelated with the spontaneous activity; however, neither requirement seems fully to be met in EEG evoked responses generally or in evoked-response audiometry (ERA) specifically. Correlated waveforms imply common Fourier spectral components, and it is recognised that a linear filter method like averaging could only achieve limited success in separating two such waveforms since it requires the waveforms to have different spectral distributions[2], in which case, averaging reduces the additive background due to components with frequency outside the spectral band in which the main power of the response waveform occurs. When all consequent improvement has been made in this way, the response waveform may still seem to be degraded by the presence of background activity within the same spectral band, and linear filter methods cannot assist further; the following remarks refer to this situation[3].

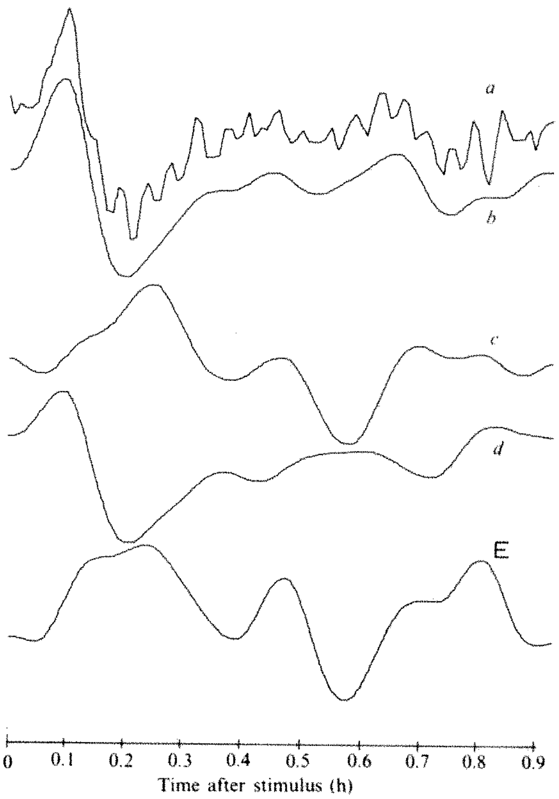

Fig. 1 Average EEG auditory-evoked responses (forty-eight stimuli, one subject). *a*, Averaged post-stimulus (response) waveform with suprathreshold stimuli (+30 dB approximately). *b*, Response waveform as in *a*, low-pass filtered to ten harmonics only. *c*, Typical averaged post-stimulus waveform obtained with subthreshold stimuli (−30 dB approximately). *d*, The waveform of *c*, phase modified according to the phase spectrum of a typical high stimulus level average response. *e*, The waveform of *c*, modified according to the amplitude spectrum of the same typical high-level response.

It is usually assumed that the ERA response consists of an additive contribution to the spontaneous activity, and this apparently unverified assumption needs examination. We have studied the ERA responses in a number of subjects using several trials with each subject at different supra- or subthreshold auditory stimulus levels; each trial used sixty auditory stimuli such as 1 kHz tone bursts (each of 60 ms duration with 10 ms rise and decay times), with constant interstimulus interval 1.25 s, at a loudness level fixed within ±30 dB of the approximate threshold for the stimulus used, and an ensemble of sixty signals obtained, each of 0.94 s length, starting from 50 ms after the stimulus. The analysis was effected by digital computer; the original signal was low-pass filtered to 50 Hz and sampled at 10 ms intervals with eleven-bit resolution (1 in 4,000) and all computations retain 10 ms intersample spacing. Ensemble averaging produced the averaged signal, and the characteristic response to a high level stimulus is shown in Fig 1*a* in wide band form, and Fig. 1*b* when filtered to ten harmonics. High-level stimuli thus produce post-stimulus averaged responses with clearly recognisable features.

Analysis suggests that, at least for moderate stimulus levels, the average power in the post-stimulus averaged signal, where a response can be seen, is not significantly different from that in a corresponding length of signal recorded and averaged from the immediate pre-stimulus period, where no response occurs. A similar finding applied to the comparison between post-stimulus averaged signals from the same individual using in one case high-level and in the other low-level stimuli (above or below threshold as evidenced by the appearance of recognisable features in the post-stimulus averaged signal). Hence a moderate-level stimulus that is effective in evoking an evident response apparently does not do so by contributing an obvious additive component. It therefore follows that the ERA response reflects some reorganisation of the spontaneous activity, effected by the stimulus. This could occur in either of two ways; by a redistribution amongst available spectral components of the total signal power in the spontaneous activity, or by a modification of the relative phases of the spectral components present in the spontaneous activity. The former would produce a difference between the amplitude spectra of post-stimulus averaged signals obtained with high-level stimuli and those with low-level stimuli; the latter way could produce a difference only in the phase spectra.

Amplitude and phase spectra were evaluated by digital-computer analysis of the averaged signals following effective high-level stimuli and of those following ineffective, low-level, stimuli; records from three adults and three children were used and the spectra were determined at all forty-seven harmonics of the fundamental sinusoid (1 cycle per 94 points = 1.06 Hz, fixed by the total post-stimulus signal length), and phases were calculated over the full 360 degree range. The amplitude values shown in Fig. 2 (one subject: four trials with high-level stimuli, five trials with low-level stimuli) typify the magnitudes and variation seen in all cases; neither from inspection nor from quantitative analysis can any difference be confirmed, for any harmonic, between amplitude spectral magnitudes of averaged signals that follow high-level (effective) or low-level (ineffective) stimuli. But phase spectral values (Fig. 3) do show a marked difference in distribution between signals according to stimulus level, low or high, particularly over the first ten or so harmonics. In the case of low-level stimuli, phase values for these harmonics are distributed approximately uniformly over the range (−180°, +180°); however, with high-level stimuli, the phase magnitudes of any given harmonic of the averaged signal tend to be much less variable between trials with similarly-effective stimuli. Hence we conclude that, in these cases, effective auditory stimuli achieve a response mainly by reorganising or constraining the phase spectra of the existing spontaneous activity in the EEG.

This proposition can be tested by imposing the average phase spectral properties of responses to a high stimulus level upon the averaged signal from low-level stimuli (Fig. 1*c* shows the original waveform filtered to ten harmonics), without altering the signal in any other way. All the spectral components of the low stimulus level averaged signal are retained without change in spectral amplitudes but their phases are those of the typical high-level response. The reconstituted waveform of the phase-modified low-stimulus-level averaged signal should then show the general features characteristic of responses to high-level stimuli typified by Fig. 1*b*. Figure 1*d* shows that this does occur. We have observed in all the cases studied so far that no other change is necessary with any of the low stimulus-level averages to transform these into an acceptable version of the high stimulus-level response, such as Fig. 1*b*; also applying this operation to any high stimulus-level response does not influence its waveform appreciably. On the other hand, if the amplitude spectrum averaged from high-stimulus level responses is imposed on the averaged signal obtained with low-level stimuli, Fig 1*c*, without allowing any change in phase spectrum, no such effect occurs, as shown in Fig. 1*e*.

Since the averaged signal in the case of the low stimulus level apparently comprises spontaneous EEG activity alone, these results also are consistent with the above proposal that, at least with some subjects, effective stimuli act by synchronising or controlling the phases of spectral components of the spontaneous EEG activity already present. It would follow that imposing the average phase-spectral properties of high stimulus-level responses upon lengths of arbitrarily selected spontaneous EEG should also be sufficient to produce a waveform acceptably similar to a true high-level response; we have found this to be true in records of control EEG from these subjects. The effect of this phase-modification on 0.94 s lengths of spontaneous EEG (selected at 1.25 s intervals from a control EEG recorded during a stimulus-free period) is shown by the correlation coefficient, *r*, calculated between an individual record length of control EEG and a reference high-level averaged evoked response. Averaged over forty-eight record lengths, $\bar{r}_{C}=0.01$ indicated, as expected, no correlation, but when the same forty-eight record lengths of control EEG were subjected to the phase modification described above (using the average phase spectrum drawn from the high-level responses for that subject), $\bar{r}_{CP}=0.63$; this shows quantitatively the substantial similarity to a true high-level response achieved by appropriate phase-spectral control imposed on arbitrary lengths of spontaneous EEG in the absence of any stimulation. These figures can be compared with correlation coefficients $\bar{r}_{S}=0.29$ and $\bar{r}_{SP}=0.70$, referring respectively to the average correlation between the same reference high-level response and forty-eight individual sweeps of record following high-level stimuli first, as recorded, and second, after phase modification of each individual sweep. Comparable figures are obtained with each subject, although of course the result would have been slightly less marked if we had allowed the phase spectral values at each harmonic to be distributed randomly around the mean value. For example, if a uniform distribution is chosen, extending to ±50 degrees from the mean, then $\bar{r}_{CP}$ (against phase-modified control EEG)$=0.43$ and $\bar{r}_{SP}$ (against phase-modified stimulated responses)$=0.39$.

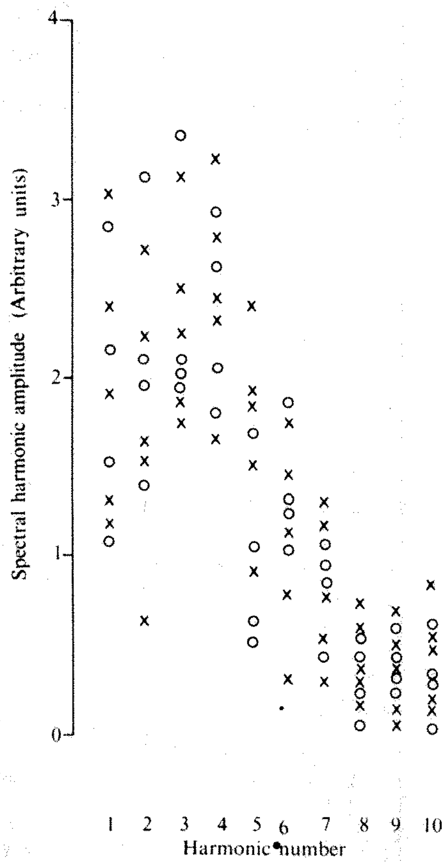

Fig. 2 Amplitude spectra of the averaged post-stimulus waveforms in nine different stimulus-level situations, four with effective, suprathreshold stimuli (○), and five with ineffective, low-level stimuli (×).

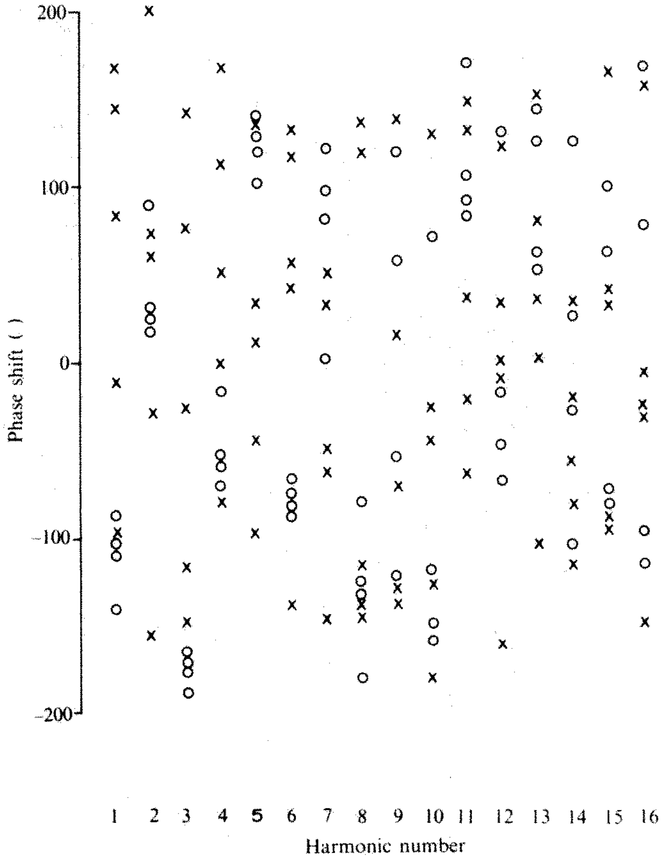

Fig. 3 Phase spectra of the waveforms as in Fig. 2.

As would be expected from these findings, it has proved possible to produce a waveform again similar ($\bar{r}=0.69$) to the high level response of one subject, by imposing the phase control appropriate to that subject on the spontaneous control EEG from another subject drawn from our series, even when the high-level responses of the two subjects (one adult, one child) are sufficiently different as to be readily distinguishable visually and very poorly correlated ($r=0.01$); the converse has also been demonstrated. If this finding is more generally confirmed, it follows that alternative methods of detecting the effect of a stimulus acting through such a mechanism may be more

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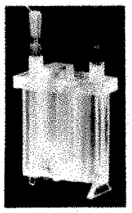

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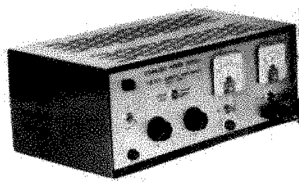

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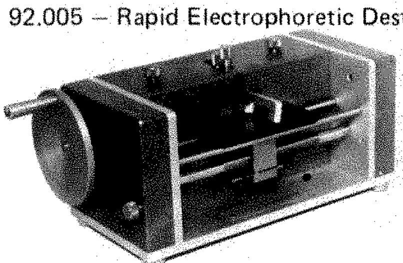

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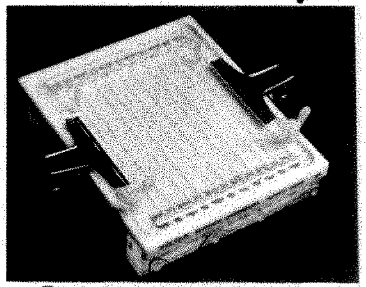

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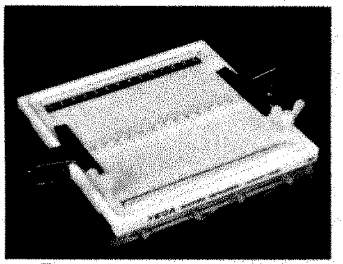

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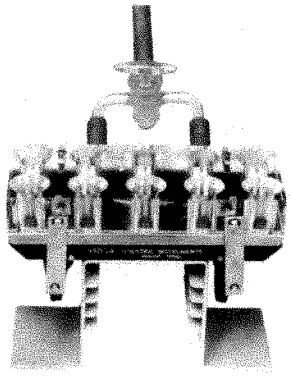

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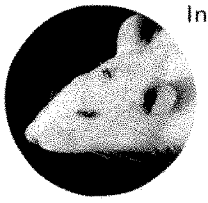

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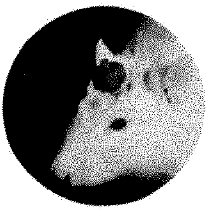

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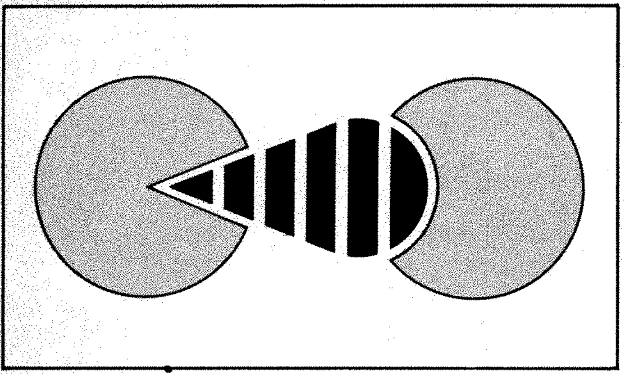

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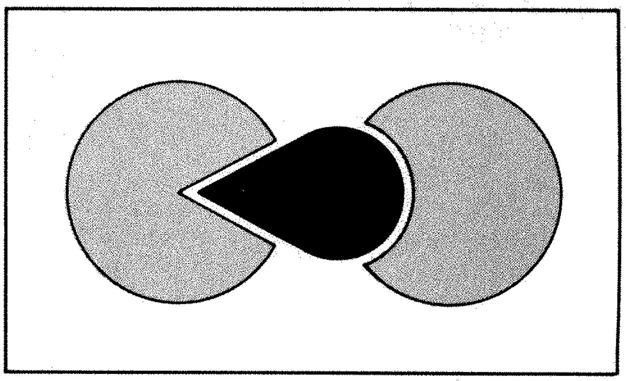

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appropriate than the usual averaging procedure; a method based on the evaluation of phase spectra in individual sweeps will be reported elsewhere. It also follows that the notion of signal-to-noise ratio (which in any case relates only to signal power, or signal amplitude) applied to a response waveform and its apparent noise background, is inapplicable to a situation in which a phase-control mechanism operates; effectiveness of a stimulus would better be expressed as the extent of phase control imposed on the spontaneous activity, and this is a readily quantifiable measure. While averaging or any other form of linear filtering will not improve the detectability of a stimulated response in the presence of additive background activity in the same spectral band, the results reported here suggesting that an effective stimulus acts mainly to phase-control the spontaneous activity, thus indicate that the approach proposed above may be fruitful. Closer attention to phase characteristics may be useful in other areas of signal processing, as for example in recognising patterns in background noise.

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Correlation of Behavioural Differences in Three Strains of Mice with Differences in Brain Amines

Much experimental evidence supports the view that brain amines may be related to behaviour, but more data are necessary regarding the relative roles of dopamine (DA), noradrenaline (NA) and serotonin (5-hydroxytryptamine) (5HT). The behavioural states have been mainly investigated using either drugs which modify catecholamine metabolism[1–12] or conditioning in which stress cannot be excluded[13].

The use of inbred strains of mice may be of particular interest for further elucidation of the neurochemical correlates and their possible relationship to acknowledged behavioural differences.

The reason for using inbred strains is the behavioural homogeneity of individuals belonging to the same strain[14], in contrast to the large difference in the behavioural traits which characterise a heterogenous population of different strains. Major differences were found in emotionality, learning, activity and aggressiveness in different strains[14–17]. Inbred strains also represent useful material in pharmacogenetic analysis of different psychoactive drugs. In spite of much comparative behavioural and psychopharmacological data, there have been few neurochemical investigations of regional brain neurotransmitter metabolism in selected strains[15,17–21].

The present study was performed with 2 to 3-month-old male mice of three strains C57Bl/6J, DBA/2J and SEC/1ReJ and their F_1 hybrids obtained from Jackson Laboratory, Bar Harbor, Maine. Previous behavioural studies performed with animals of these strains of similar age and sex to those used here have shown that strain differences exist in wheel-running activity, avoidance behaviour and maze learning[22].

The C57Bl strain is characterised by high spontaneous activity (304.5 wheel revolutions h^{-1}) and low levels of avoidance (6.6% avoidance in a shuttle box and 94 errors in a maze), while the two other strains (DBA and SEC) attained high avoidance levels (respectively 40 and 53.4% avoidance and 69.3 and 50.5 maze errors), but much lower general activity (respectively 230.3 and 203.6). Crossing the C57 strain with the high avoiding low running SEC mice resulted in SEC-like behaviour progeny (activity 195, avoidance 48.3%, maze learning 46.9), while crossing the C57 strain with the high-avoiding low-running DBA mice yielded offspring similar to the C57 phenotype (activity 288.1, avoidance 8.3% and maze learning 45.9). Behavioural data are from Oliverio *et al.*[22].

This report concerns catecholamine metabolism in whole brain and some areas of the central nervous system in the three strains.

The mice were killed by decapitation. The total brain and the different areas (hypothalamus, pons medulla and cortex) (Fig. 1) were removed, weighed, frozen and stored in liquid nitrogen.

The tissues were homogenised in 10 vol of acid ethanol (ethanol-water-HCl 0.05 N, 74:16:10 (v/v) at 0° C in a Potter Elvehjem homogeniser and then centrifuged at 10,000*g* for 10 min. The amines were separated on columns of Amberlite CG50 200–400 mesh[23]. Noradrenaline[24], dopamine[25] and 5HT[26] were estimated fluorometrically. Turnover parameters were determined by blocking the synthesis of noradrenaline and dopamine by intraperitoneal injections of α-methyltyrosine (DL-α-methyl-*p*-tyrosine methylester HCl, AB Biotec Stockholm, Sweden) (300 mg kg^{-1}) according to Brodie *et al.*[27]. Animals were killed 1–3 h after injection. Breakdown of 5HT was blocked with pargyline (75 mg kg^{-1}) and the subsequent changes in 5HT concentrations were determined[28]. Regression lines were calculated from the least squares fit. The results were analysed statistically by analysis of the covariance[29].

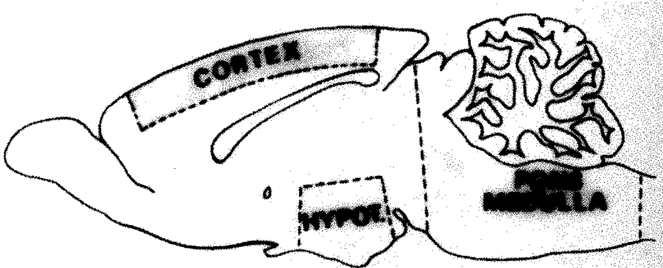

Fig. 1 Diagrammatic representation of dissection procedure for mice brain.

The weights of the mice and their brain weights are given in Table 1. The amount of NA is significantly higher in the whole brain of C57 mice compared with DBA and SEC

Table 1 Mean values for body and total brain weights in three strains of mice

	Mouse weight g±s.d.	Brain weight mg±s.d.
C57Bl/6J	26±1.7	451±17
DBA/2J	26±1.5	390±15†
SEC/1ReJ	23±1.2*	427±17†

* $P < 0.02$. † $P < 0.001$.

(Table 2). After α-methyltyrosine injection (Table 2), the slope of the decrease of NA is rather close in the three strains, as is also the turnover time. Since the amount of NA is higher in the C57, the turnover rate is also slightly higher.

Table 2 Brain noradrenaline concentrations and turnover in three strains of mice

	NA (ng/gww ± s.d.)	(ng/brain ± s.d.)	After α MT (slope ± s.d.)	Turn-over time (h)	Turn-over rate (ng/g/h)
C57Bl/6J	589 ± 40 (29)	266 ± 19	0.077 ± 0.007	5.6	104.5
DBA/2J	562 ± 45 * (29)	219 ± 17 †	0.072 ± 0.006	6.0	93.2
SEC/1ReJ	535 ± 38 † (25)	228 ± 17 †	0.080 ± 0.006	5.4	98.5

* $P < 0.05$.
† $P < 0.001$.
Numbers in parentheses represent the number of mice.

In contrast to NA, the dopamine content per g whole brain are significantly greater in DBA and less in SEC compared with C57 (Table 3). When expressed in ng per brain, there were no differences between the C57 and DBA mice; only SEC presenting significantly lower values. The slope of the decrease of DA after α-methyltyrosine injection is significantly different between the three strains; as is the turnover time and the turnover rate.

Table 3 Brain dopamine levels and turnover in three strains of mice

	DA (ng/gww ± s.d.)	(ng/brain ± s.d.)	After αMT (slope ± s.d.)	Turnover time (h)	Turnover rate (ng/g/h)
C57Bl/6J	1,559 ± 189 (29)	703 ± 91	0.097 ± 0.008	4.5	348
DBA/2J	1,763 ± 216 * (28)	687 ± 82	0.077 ± 0.008 *	5.6	313
SEC/1ReJ	1,353 ± 193 * (25)	581 ± 88 *	0.137 ± 0.009 *	3.2	427

* $P < 0.001$. Numbers in parentheses represent numbers of mice.

The amount of serotonin per g fresh weight is similar in the three strains (Table 4), however the quantity per brain is lower in DBA and SEC compared with C57 mice. After pargyline injection the slope of the increase of 5HT is very similar in the three strains, as is also turnover time. Since the amount of 5HT in brain is higher in C57 strain the turnover rate seems to be slightly higher in C57 mice.

When pons medulla oblongata, hypothalamus and cortex were analysed separately (Table 5), a significantly higher amount of NA was found in pons medulla in the C57 strain compared with DBA and SEC.

In contrast, less NA was found in the hypothalamus of C57 mice. In the cortical zone markedly decreased amounts were found in the SEC brain compared with C57 and DBA, the latter two having similar values.

When determining the turnover parameters of NA in the different brain areas only he slope of the decrease of NA in the pons medulla of C57 mice is significantly different from DBA and SEC. The turnover time is much quicker in the pons medulla of the C57 mice.

Table 4 Brain serotonin levels and turnover in three strains of mice

	5 HT (ng/gww ± s.d.)	(ng/brain ± s.d.)	Slope ± s.d.	Turn-over time (h)	Turn-over rate (ng/g/h)
C57 BL/6J	1,041 ± 97 (22)	472 ± 45	3.21 ± 0.84	5.43	192
DBA/2J	1,016 ± 95 (22)	397 ± 38 *	3.08 ± 0.84	5.50	185
SEC/1ReJ	1,017 ± 73 (18)	431 ± 26 *	2.98 ± 0.83	5.67	179

* $P < 0.001$.
Numbers in parentheses represent numbers of mice.

In the F_1 generation (Table 6) crossing DBA and C57, values for NA in pons medulla closer to C57 were obtained, but crossing SEC and C57, values closer to SEC were observed. In the hypothalamus the NA levels were closer to those of C57 in both crosses C57×DBA and C57×SEC, while in the cortical area, the value from C57/DBA was lower than in the parental strain and in C57/SEC close to the value from parent SEC.

As transmitters are localised in specific areas, determination of the catecholamines in terms of ng per g whole brain may be misleading. Since the brain weight is different in the three strains seemingly high values will be obtained for animals having lower brain weights. The catecholamine levels for whole brain are more suitable for evaluating the steady state situation.

The higher levels of NA in the whole brain and in the pons medulla in the C57 strain may be correlated with the greater activity of these mice as shown in Fig. 2, *a* and *b*. Similarly, the turnover rate which is higher in C57 than in DBA and SEC may also agree with the results of the activity test. Apparently lower values of NA in pons medulla where the reticular formation is localised, agree well with the lower activity of DBA and SEC strains as well as with the activity behaviour of the F_1 generation crossing either C57 and DBA or C57 and SEC (Fig. 2, *a* and *b*).

Table 5 Regional brain noradrenaline turnover in three strains of mice

		NA Steady-state level (ng/g)		NA decline after αMT (slope ± s.d.)	Turn-over time (h)	Turn-over rate (ng/g/h)
Pons medulla oblongata	C57	802 ± 43	(20)	0.086 ± 0.009	5.02	160
	DBA	700 ± 38 †	(20)	0,071 ± 0.009 †	6.13	114
	SEC	740 ± 62 †	(14)	0.075 ± 0.007 *	5.75	128
Hypo-thalamus	C57	1,468 ± 202	(20)	0.074 ± 0.011	5.88	250
	DBA	1,648 ± 178 *	(20)	0.073 ± 0.013	5.95	277
	SEC	1,634 ± 98 *	(16)	0.074 ± 0.011	5.88	277
Cortex	C57	526 ± 44	(16)	0.077 ± 0.008	5.58	94
	DBA	552 ± 71	(16)	0.082 ± 0.009	5.32	104
	SEC	375 ± 73 †	(8)	0.086 ± 0.013	5.03	75

* $P < 0.01$.
† $P < 0.001$.
Numbers in parentheses represent numbers of mice.

The higher values for hypothalamus NA obtained in DBA and SEC are interesting, since this area is involved in several behavioural states. A correlation with the avoidance and maze learning performance is more difficult to establish. A correlation between hypothalamic NA content and avoidance behaviour (Fig. 2*c*), and cortex NA content and maze learning (Fig. 2*d*) can be proposed, however, in the C57/

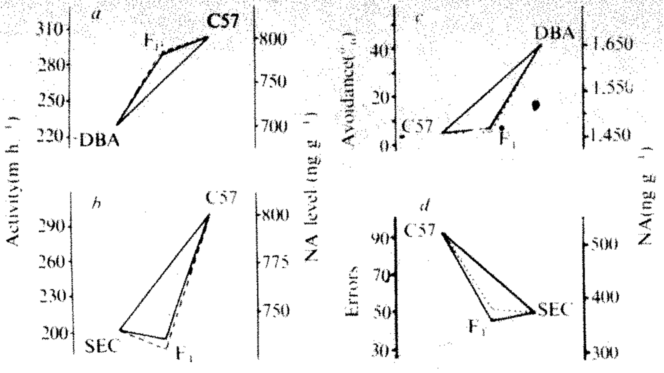

Fig. 2 Correlation between performances and NA concentrations in parental strains and their F_1 hybrids. *a–b*, Correlation between activity and NA level in pons medulla; *c*, correlation between avoidance and NA level in hypothalamus; *d*, correlation between maze learning and NA level in cortex. Unbroken lines, performances (compare Oliverio[22]); broken lines, NA level in the different areas of brain.

Table 6 Noradrenaline in various brain areas of three strains of mice and their F_1 hybrids

		DBA		C57		SEC
Pons medulla oblongata	Parental	700 ± 38		802 ± 42		740 ± 62
	F_1		779 ± 43		728 ± 42	
Hypothalamus	Parental	1,648 ± 178		1,468 ± 202		1,634 ± 98
	F_1		1,473 ± 102		1,450 ± 143	
Cortex	Parental	552 ± 71		526 ± 44		375 ± 73
	F_1		470 ± 19		383 ± 39	

DBA cross for avoidance behaviour and in the C57/SEC cross for maze learning. It must be pointed out that the NA concentration in the hypothalamus of the F_1 offspring from a C57/SEC cross is identical to that of C57, whereas in the two other areas (cortex and pons medulla) the parent SEC characteristic is dominant.

It is interesting to note that for the whole brain, there is also significantly less DA in SEC, and for DBA the values are very close to C57.

Finally the whole brain 5HT content is also significantly higher in the C57 strain.

We conclude from these data that (1) the NA steady state concentrations in whole brain and some areas such as cortex, hypothalamus and pons medulla are significantly different in different inbred mice strains; and (2) heritability of NA level is similar in pons medulla and hypothalamus in the F_1 generation offspring from C57/DBA cross and these values are close to those of the C57 parent. In the F_1 generation offspring, from C57/SEC cross the NA level is close to that of the SEC parent in pons medulla and cortex but not in the hypothalamus.

Thus a correlation seems to exist between the behavioural phenotype and NA levels of cerebral regions. In particular there is a high correlation between pons medulla NA level and the activity of the C57, DBA and SEC mice and their F_1 hybrids.

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Aerosol Chemotherapy of Lung Neoplasia

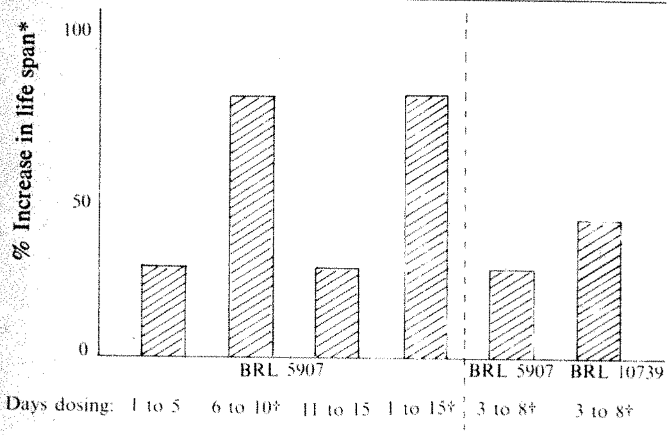
The administration of anticancer drugs in amounts necessary to obtain a therapeutic response can be associated with severe toxicity. Attempts to circumvent such toxicity have been made and examples of this include intra-arterial infusion chemotherapy and the administration of drugs directly into neoplastic lesions. We wish to suggest a novel extension of such regional cancer chemotherapy and to describe the use of aerosol dosing to confer protective antitumour effects in the lungs of mice carrying certain transplanted tumours.

The materials used in this study were the double-stranded RNA, BRL 5907, isolated from a mycophage, and a ribonuclease resistant complex of this nucleic acid BRL 10739 (ref. 1). Solutions of these nucleic acids were nebulised into fine droplets using a Wright CF1 aerolyser (Aerosol Products

(Colchester) Ltd). The particle sizes were less than 8 μm in diameter and the substantial proportion was about 1 μm. These droplets were therefore able to penetrate to the lower respiratory tract including the alveoli of the lungs[2]. Mice bearing a transplantable tumour were placed in a chamber and exposed to the aerosol. The drugs, which are macromolecular and not orally absorbed, were inhaled by the mice and partly retained in the lung. The dose which was retained was calculated from the volume of the lung and respiration rate and using a 30% retention value as previously reported[3]. The dose can be adjusted by changes in the concentration of the drug, the flow of aerosol through the chamber and time of the experiment.

The FS6 mouse tumour, a benzpyrene-induced fibrosarcoma, was the first model to be studied[4]. FS6 tumour cells (0.1 ml trypsinised suspension of 10^5 cells) were injected by the intravenous route into syngeneic C57Bl/6 mice. The cells lodge in the capillary bed of the lung and grow to form tumours which kill the animal after three to four weeks. In the first experiment BRL 5907 was given as an aerosol at a dose of 0.4 mg kg^{-1} d^{-1} to mice bearing the FS6 tumour at different times over the first 15 d after transplantation (Table 1).

Table 1 Effect of BRL 5907 and BRL 10739 by the aerosol route on the FS6 fibrosarcoma



* Calculated on day of median death in groups of ten mice.
† Increase in survival time compared with controls were statistically significant ($P<0.01$). 10^5 cells given intravenously on day 0. Drugs given in an aerosol chamber 0.4 mg kg^{-1} d^{-1}. Controls were dosed with aerosol saline, median day of death 30.

An increase in survival time over the controls was found and this varied, depending upon when the drug was given. The optimum response of a 73% increase in survival time was observed when dosing from day 6 to day 10 after inoculation. Thus the median day of death in this experiment was increased from 30 d to 50 d and there were four survivors in this group of ten mice. Weaker antitumour effects were obtained if the drug was given either earlier or later. Giving the drug for 15 d was no more effective than giving it between day 6 and day 10 after tumour inoculation. In a further experiment, BRL 5907 was compared with BRL 10739 again at a dose level of 0.4 mg kg^{-1} d^{-1}. An increase in life span of 26% was found with BRL 5907 when dosing on day 3 to day 8 and this compared with a value of 38% with BRL 10739.

We also compared the low dose double-stranded RNA aerosol with systemic administration. Intraperitoneal doses of BRL 5907 and BRL 10739 had to be increased to 2.5 mg kg^{-1} d^{-1} to show even the relatively low increase in survival times of 24% and 28% respectively when compared with controls (Table 2). It seemed that a low level of drug applied to the lung gave a better therapeutic effect in the lung than larger amounts given by a systemic route. It is difficult to give larger doses of these double-stranded RNAs by aerosol but it is possible to use intranasal drops. BRL 5907 (5.0 mg kg^{-1} d^{-1}) and BRL 10739 (2.5 mk kg^{-1} d^{-1}) given by intranasal drops on days 3, 5 and 10 after inoculation showed an increase in survival time over control FS6 mice of 53% and 47% respectively.

Table 2 Effect of BRL 5907 and BRL 10739 by the intranasal and intraperitoneal route on the FS6 on the fibrosarcoma

Drug	Route	Dose mg kg^{-1}	Schedule days of dosing	Increase in survival time %*
BRL 5907	Intranasal drops	5.0	3, 5, 10	53†
	Intraperitoneal	2.5	4, 8, 10, 13, 15, 17	24
BRL 10739	Intranasal drops	2.5	3, 5, 10	47†
	Intraperitoneal	2.5	4, 8, 10, 13, 15, 17	28

* Calculated on day of median death in groups of ten mice.
† Increase in survival time compared with controls were statistically significant ($P<0.001$). 10^5 cells given intravenously on day 0.

We were interested to confirm this aerosol activity in another tumour system. The Lewis lung carcinoma of the mouse was chosen because we could study the effect of these nucleic acids given by aerosol on the pulmonary metastases ensuing from a primary tumour present in the host[5]. The tumour was transplanted subcutaneously as small fragments and grew to some 3.0 g over 21 d. The mice were then killed, the tumour excised and weighed and the lungs examined for metastases[6]. BRL 5907 and BRL 10739 were given as an aerosol at a dose of 0.4 mg kg^{-1} d^{-1} for 5 d each week to mice bearing the Lewis lung tumour.

Table 3 Effect of BRL 5907 and BRL 10739 by the aerosol route on the Lewis lung carcinoma

Drug	% tumour inhibition	Average number of lung metastases
BRL 5907	0	1.0 large 3.0 small ($P<0.025$)*
BRL 10739	0	0.5 large 2.5 small ($P<0.01$)*
Saline controls	—	1.0 large 10.0 small

Tumour implanted day 0, subcutaneously into the flank of groups of eight mice. Drugs dosed by aerosol 0.4 mg kg^{-1} d^{-1} on days 2 to 4, 7 to 11, 14 to 18.
* The number of metastases were significantly different when compared with controls.

As expected, there was no inhibition of the primary tumour (Table 3). There was, however, a definite effect against the lung metastases and this was shown by a greater than 50% reduction in the average number of metastases in the lungs of treated animals when compared with controls. At the dose and schedule used, there was no apparent difference between the two drugs. When these drugs were given at the same dose, and on the same days, by the intraperitoneal route there was no difference between the treated and control groups. Thus, we have again shown an advantage in antitumour effects in the lung when giving low aerosol doses compared with systemic administration.

The treatment of primary and metastatic lung cancer in man with anticancer drugs it notoriously ineffectual. A possible reason for this is that the surface area of the lung is very large and the concentration of a drug at any point within the lung could be too low to deal with the malignant cells. Any attempt to raise this concentration by higher doses inevitably brings added systemic toxicity. We have demonstrated antitumour activity in the lungs of mice given low aerosol doses of antitumour drugs. We suggest that this could be a novel and useful approach to the chemotherapy of pulmonary malignant disease in man enabling high concentrations of drug to be introduced in the lung.

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Cumulative Cytostatic Effect of ICRF 159

THE antimitotic action of (±)-1,2-bis (3,5-dioxopiperazin-1-yl) propane (ICRF 159) has been extensively studied *in vivo* in human[1] and animal tumours[2,3], but its mode of action at the cellular and biochemical level has not yet been elucidated. Although it reduces the rates of DNA, RNA and protein synthesis of cells *in vitro*[4], it has also been shown to block the cell generation cycle in the post-DNA synthesis (G_2) phase[5,6]. Tumours whose growth is halted contain many large multinucleate cells not seen in control tumours. An attempt has therefore been made to see if the morphological changes produced by the drug in cells grown *in vitro* could provide an indication of its intracellular site of action and point towards possible mechanisms of action. The cells used were non-synchronised cultures of non-transformed hamster fibroblasts (Nil 8 cells), HeLa cells and fibroblasts derived from a transplantable fibrosarcoma in Syrian hamsters (T17 cells). They were exposed to single daily doses of ICRF 159 for 3 d.

For the first 24 h, growing cultures of Nil 8, T17 and HeLa cells in concentrations of ICRF 159 up to 20 μg ml^{-1} increased in number at the same rate as the controls. Between 24 to 48 h, however, the rate of cell increase was reduced by the drug in inverse proportion to its concentration. Between 48 to 72 h, cell numbers continued to increase in concentrations up to 10 μg ml^{-1} (Table 1). At the same time an analysis of the distribution of cell sizes using a Coulter counter showed that Nil 8, T17 and HeLa became bigger with increasing time in increasing concentrations of ICRF 159 (Fig. 1).

Table 1 The effect of ICRF 159 on three cell lines

Cell lines	Hours of culture	Numbers of cells (× 10^5) per dish in medium containing ICRF 159 0 μg ml^{-1}	2.5 μg ml^{-1}	5 μg ml^{-1}	10 μg ml^{-1}	20 μg ml^{-1}	40 μg ml^{-1}
Nil 8	24	2.5	2.5	2.5	2.5	2.3	2.0
	48	5.5	5.0	3.0	2.7	1.7	1.0
	72	16.2	12.1	10.0	1.5	1.4	0.9
T17	24	2.5	2.5	2.5	2.3	2.0	2.0
	48	8.7	7.0	4.2	4.0	3.5	2.0
	72	21.4	12.0	5.3	2.0	1.9	1.5
HeLa	24	2.9	2.9	2.5	2.0	2.5	2.0
	48	8.0	6.2	4.5	3.5	3.0	1.5
	72	15.0	12.4	10.0	3.0	2.5	1.0

The cells were plated at 1×10^5 per 5 cm dish at 0 h, ICRF was added 90 min, 24 and 48 h later. The cells were trypsinised from the dishes and their number counted in a haemocytometer. The results are derived from duplicate experiments using three dishes per experimental point.

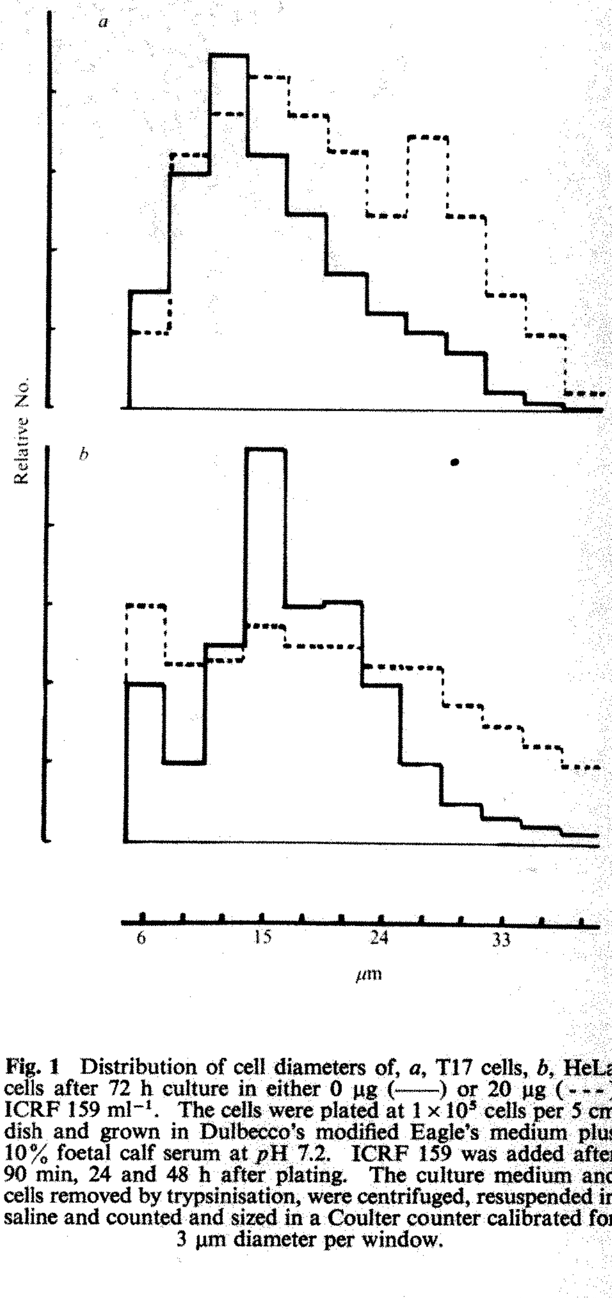

Fig. 1 Distribution of cell diameters of, *a*, T17 cells, *b*, HeLa cells after 72 h culture in either 0 μg (——) or 20 μg (- - -) ICRF 159 ml^{-1}. The cells were plated at 1×10^5 cells per 5 cm dish and grown in Dulbecco's modified Eagle's medium plus 10% foetal calf serum at pH 7.2. ICRF 159 was added after 90 min, 24 and 48 h after plating. The culture medium and cells removed by trypsinisation, were centrifuged, resuspended in saline and counted and sized in a Coulter counter calibrated for 3 μm diameter per window.

Morphological studies confirmed that Nil 8, T17 and HeLa cells became larger when exposed to concentrations of ICRF 159 greater than 5 μg ml^{-1} for 24 h or longer and showed that numbers of bi- or multinucleate cells appeared in the cultures. These changes were most noticeable after 72 h culture in 20 μg ml^{-1}. HeLa cells differed from Nil 8 and T17 cells in that a significant proportion of dead HeLa cells were seen (dye-exclusion test) in concentrations of ICRF 159 that produced few if any dead Nil 8 or T17 cells. The dead cells were predominately small mononuclear cells. Other workers in these laboratories have shown that cultured BHK-215 cells became multinucleated when treated with ICRF 159 and by time-lapse cinematography have demonstrated that this is the result of an interference with the separation of daughter cells (T. C. Stephens and A. M. Creighton, unpublished).

Ultrastructural study, however, showed that increasing cell size was not accompanied by osmotic or degenerative changes in Nil 8 and T17 cells although a few of the HeLa cells contained cytoplasmic hydropic vacuoles. Surprisingly, the large mono-, bi-, or multinucleate cells contained normal-looking cell organelles (Fig. 2). The numbers of mitochondria and ribosomes were increased in proportion to other cytoplasmic organelles, the nuclei of the large cells were often deeply folded and nucleoli were mostly present. Occasional mitotic figures were seen, the centrioles and spindle tubules were normal both in the small and large cells, but the appearance of the centriole in some cells suggested that they were in G_2 (ref. 7). This may be the point at which the dividing cells are blocked as has been previously found[5]. Although centrioles were often present in cells from control cultures, none resembled those seen in G_2. Occasional cells in control and ICRF 159-treated cultures had both electron-dense nuclei and cytoplasmic organelles and peripheral cytoplasmic projections. They were most abundant in HeLa cells after 72 h in 20 μg ml^{-1} ICRF 159. These cells resembled apoptotic cells[8].

Table 2 Labelling index of T17 cells after culture in ICRF 159

(µg ml)	Culture (h)	Increase in cell number (×)	Labelling index
0	0 to 24	2.5	80±3
	24 to 48	2.8	78±3
	48 to 72	2.4	79±2
	0 to 72	16.0	90±3
5	0 to 24	2.5	88±2
	24 to 48	1.8	70±2
	48 to 72	1.1	70±2
	0 to 72	5.2	85±3
20	0 to 24	2.1	82±2
	24 to 48	1.5	59±2
	48 to 72	0.7	59±2
	0 to 72	2.2	85±4

The cells were plated at 1×10^5 at 0 h and ICRF 159 and 0.2 µCi/methyl-^{3}H thymidine were added 90 min, 24 and 48 h later. After trypsinisation, the cells were processed as for electronmicroscopy (Fig. 2). Sections 0.5 µm thick, were mounted on glass, dipped in NTE K5 emulsion, exposed for 1 to 7 d and developed in Kodak D19. 500 Cells were counted for each point and the results are derived from triplicate experiments.

The incorporation of methyl-^{3}H thymidine in the presence of ICRF 159 was studied in T17 cells (Table 2). The labelling index of both control and ICRF 159-treated cells was similar during the first 24 h whereas the index was greater in control cells than in treated cells during the 24 to 48 and 48 to 72 h periods. Cells cultured for up to 72 h in even 20 μg ICRF 159 ml^{-1} were still capable of thymidine incorporation although the concentration was sufficient to inhibit cell division. Moreover, the intensity of nuclear labelling (grains per nucleus) was similar in cells from control and drug-treated cultures during 0 to 24, 24 to 48 and 48 to 72 h periods. The multilobed nuclei were as capable of DNA synthesis as were the nuclei of mononuclear cells. Where a binucleate cell was labelled, both nuclei had similar grain counts. A high proportion of unlabelled nuclei during the 48 to 72 or 0 to 72 h periods were either fragmented or of a bizarre shape.

As many of the cells cultured up to 72 h in ICRF 159 were viable and able to synthesise DNA, the relationship between length of exposure, drug concentration and cell numbers was further investigated. *In vivo* studies have shown that a single dose of ICRF 159, however large, has little or no effect on tumour growth[9], whereas quite small doses repeated on three consecutive days could produce complete inhibition (K. H., and K. Burrage, unpublished). T17 cells were therefore cultured for either 0 to 24 h, 0 to 48 h or 0 to 72 h in medium that contained 2.5 to 20 μg ICRF ml^{-1} and, after washing for 30 min with drug-free medium, were allowed to continue their growth in drug-free medium for the next 48 to 96 h. The increase in cell numbers 48 h after a 24 h exposure to the drug was slightly less than in control cultures (Fig. 3) whereas it was much less after a 48 h exposure. During incubation in drug-free medium, cell numbers increased after an initial lag period that was proportional to the dose of ICRF 159 but cell numbers showed some recovery even after exposure to 20 μg ml^{-1}. During the recovery phase the numbers of small mononuclear cells increased whereas the numbers of large mono- or binucleate cells remained fairly constant. Cultures exposed to 5 μg or more of ICRF 159 for 0 to 72 h before washing and reculturing in drug-free medium showed little, if any, recovery of cell numbers after 96 h further culture (Fig. 4).

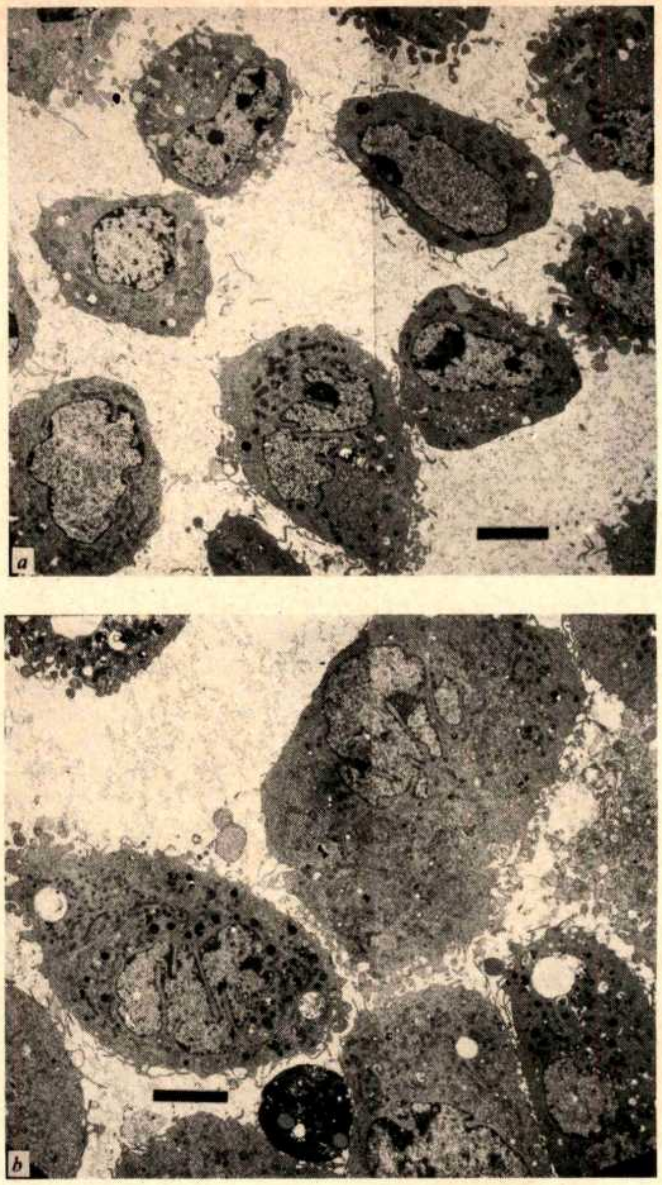

Fig. 2 Comparison of the ultrastructural morphology of T17 cells after 72 h culture in either, *a*, 0 µg or, *b*, 20 µg ICRF 159 ml^{-1}. After trypsinisation, the cells were fixed with 2.5% glutaraldehyde in Sorenson's phosphate buffer, gelled in albumin-glutaraldehyde, secondarily fixed in 1% osmic acid, dehydrated and polymerised in Araldite. The ultrathin sections were stained with methanolic uranyl acetate and aqueous lead citrate. The cells treated with ICRF 159 are larger than untreated cells, have increased numbers of normal cytoplasmic organelles and show exaggerated nuclear folding. The scale is equivalent to 5 µm.

Studies on erythroid maturation have shown that the cytostatic effect of ICRF 159 was seen only in dividing cells[10]. To confirm that no morphological changes were produced on non-dividing cells, the effect of ICRF 159 on confluent cultures of Nil 8 cells was studied as these cells show complete contact inhibition of cell divison without becoming detached

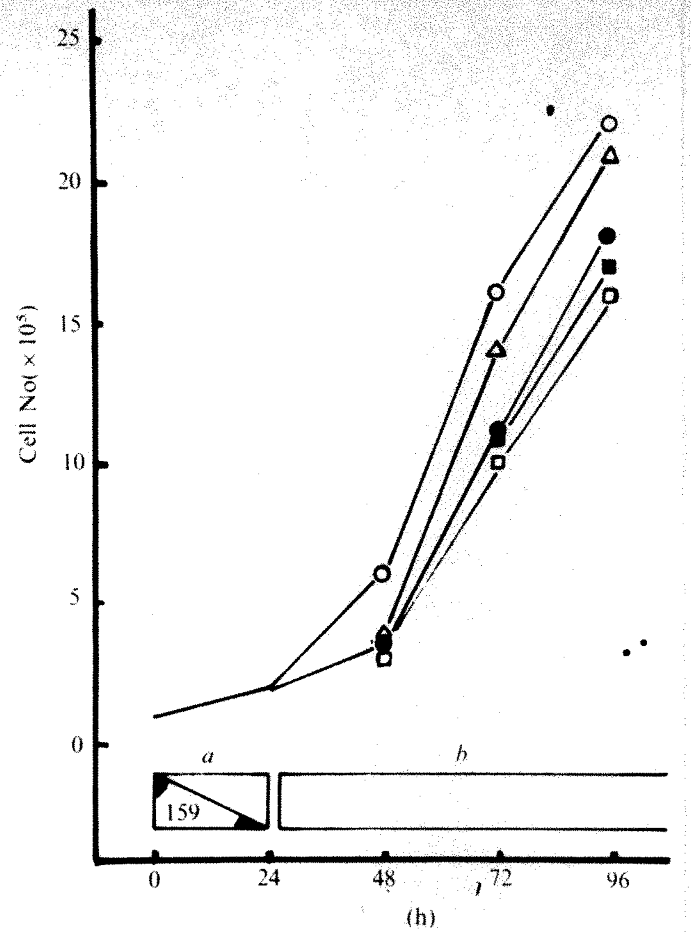

Fig. 3 Effect of 0 to 24 h culture in medium containing ICRF 159 on numbers of T17 cells. The cells were plated at 1×10^5 per dish at 0 h, ICRF 159 was added 90 min later. After 24 h culture the medium was removed and replaced with drug-free medium. The numbers of cells per dish was determined 24, 48 and 72 h later. *a*, Falling concentration of ICRF 159 due to chemical degradation in culture medium during the 24 h period; *b*, drug free medium. ○, Control; △, 2.5 µg ICRF 159 ml; ●, 5 µg ICRF 159 ml^{-1}; ■, 10 µg ICRF 159 ml^{-1}; □, 20 µg ICRF 159 ml^{-1}.

from the base of the plastic dishes. Confluent cultures of Nil 8 cells were exposed for 0 to 72 h to concentrations of ICRF 159 of either 5 or 20 μg ml^{-1}. The cells were removed from the dishes by trypsin, their numbers counted and equal numbers plated into dishes containing drug-free medium. After a further 48 h culture, no differences were found either in cell morphology or numbers per dish between confluent cells cultured with or without ICRF 159. It was possible that the degradation products of ICRF 159 accumulating in the culture medium during 0 to 72 h were inhibitory to cell growth. It was, therefore, used as the growth medium for non-confluent Nil 8 cells. The cells increased in numbers during the next 48 h at a greater rate than did similar cells in control medium. The degradation products of the drug are therefore not inhibitory to cells growing in conditioned medium.

Other chemotherapeutic agents induce the formation of mono- and binuclear giant cells in culture. Several nitrogen mustards allow DNA synthesis to continue above the premitotic level, although this treatment may result in cells blocked at either the end of S (ref. 12), S/G$_2$ (ref. 13) or during G$_2$ (ref. 14). It seems, therefore, that if nuclear DNA synthesis is permitted, but cell division is blocked at either S/G$_2$, G$_2$ or G$_2$/M, multinucleate giant cells will appear in the culture and their appearance is both dose and time dependent. The effects of ICRF 159 on cultured cells reported here are compatible with an interference with the mitotic cycle during S/G$_2$, G$_2$, or G$_2$/M.

Several conclusions clearly emerge from the present studies. The drug only acts on cells during their generation cycle. It is without effect on resting cells. The inhibitory action is proportional to the drug concentration in the medium. The inhibition is reversible after one exposure to the drug, but cumulative if the concentration is above a threshold level and given repeatedly. It is to be expected that drug concentrations and length of exposure required to produce inhibition of cell division will vary with cell types. Because of the remarkable lack of structural damage which accompanies these very subtle, but precise insults to the cell division mechanism, however, and because of doses of the drug which ultimately inhibit division, but permit the cells to go normally through DNA synthesis, the present studies on non-synchronised cells have not been able to pin-point the intracellular site or the biochemical mode of action of the drug. They are, however, in excellent agreement with earlier *in vitro* work which indicated that the block of cell division occurred at the end of G$_2$ or beginning of mitosis and with *in vivo* findings that single large doses are without effect and that at least 3 consecutive doses are required for antitumour activity. It needs to be stressed, however, that *in vivo* tumour systems appear to be much more sensitive to the action of ICRF 159 than the present *in vitro* studies would suggest.

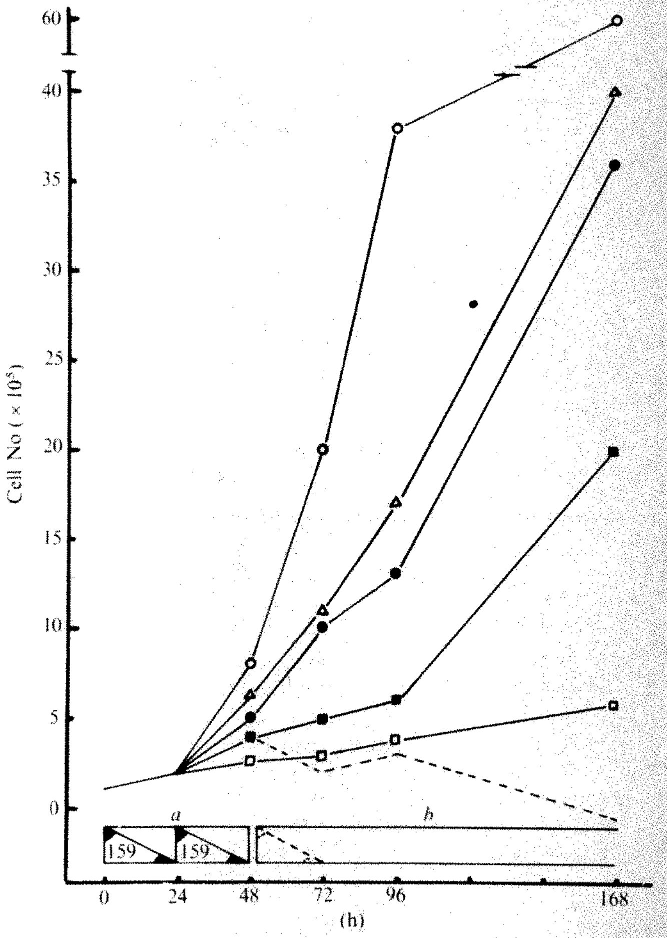

Fig. 4 Effect of 48 h culture in medium containing ICRF 159 on numbers of T17 cells. The cells were plated at 1×10^5 per dish at 0 h, ICRF 159 was added 90 min and 24 h later. After 48 h culture the medium was removed and replaced with drug-free medium. The numbers of cells per dish was determined 24, 48 and 72 h later. (- - -), additional 10 µg ICRF 159 added at 48 h and the medium changed at 72 h. *a*, Falling concentration of ICRF 159 due to chemical degradation in culture medium during the 24 h period; *b*, drug-free medium. ○, Control; △, 2.5 µg ICRF 159 ml^{-1}; ●, 5 µg ICRF 159 ml^{-1}; ■, 10 µg ICRF 159 ml^{-1}; □, 20 µg ICRF 159 ml^{-1}.

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Two-stage Malignant Transformation of Rat Fibroblasts in Tissue Culture

MOST of the *in vitro* systems that have been developed to study the mechanisms of chemical carcinogenesis use rodent fibroblasts grown in culture[1-3]. Since they are rapid and permit the control of many parameters, these *in vitro* malignant transformation techniques offer advantages over the *in vivo* procedures previously employed.

Co-carcinogenesis, which occurs in two distinct stages, those of initiation and promotion[4-7], has been demonstrated in skin[8-9], in the forestomach of the mouse[10] and in other tissues[11]. 7,12-Dimethylbenz(a)anthracene[12], benzo(a)pyrene[13] and urethane[14] have been the most commonly used initiating agents while croton oil[15] and phorbol ester[16-17], an active constituent of this plant extract, have frequently been employed as promoting substances. Analysis of the cellular changes that occur in co-carcinogenesis *in vivo*, however, is not easy; for example, metabolism that leads to the inactivation and excretion of the carcinogen and variations in the turnover of cells in the treated tissue are two of the factors that complicate quantitative investigations of co-carcinogenesis in animals. Here we report results which show that malignant transformation of rat fibroblasts grown *in vitro* can proceed in two stages, those of initiation and promotion.

The experiments were carried out with embryonic fibroblasts from Wistar rats, grown in culture and serially passaged every 5 d[18]. Cell cultures were treated with benzo(a)pyrene or with phorbol ester or with both compounds which were added to the medium as acetone solutions; other cell cultures remained untreated. Tests for cell transformation were made on the subcultures at intervals as shown in Tables 1–3. The three criteria of *in vitro* malignant transformation that were used have been described previously[18] and consist of (1) the cloning of cells in liquid medium using a feeder layer composed of irradiated cells, transformation being recognised by the abnormal morphological arrangement of the cells in the colony[3,19], (2) the cloning of cells in agar suspension; transformed cells have the ability to develop into three-dimensional colonies under these conditions[20] and (3) the subcutaneous inoculation of cells into newborn rats where transformed cells produce progressively growing tumours.

The cumulative effects of initiation with benzo(a)pyrene and promotion with phorbol ester on the incidence of morphologically transformed colonies that arose from cells cloned in liquid medium are summarised in Table 1. These results show that, until the twenty-first passage, neither initiation alone nor initiation followed by promotion for six cell passages, gave a marked increase in morphological transformation. Subsequently the incidence of colonies showing morphological transformation increased sharply in both groups of treated cells but those cells treated with both initiator and promoter showed a higher efficiency of transformation. The incidence of spontaneous transformation in the untreated cells was only significantly increased after forty passages (Table 1).

Table 1 Transformation of Cells Treated with Benzo(a)pyrene and Phorbol Ester and Cloned in Liquid Medium

	Treatment*		Transformation assay			
Passage No.	Initiation with benzo(a)-pyrene	Promotion with phorbol ester	No. of colonies	Plating efficiency (%)	No. of transformed colonies	Efficiency of transformation (%)
8	1	0	397	39.7	10	2.5
	0	0	312	31.2	4	1.4
10	1	0	258	25.8	6	2.3
	0	0	174	17.4	3	1.7
21	1	6	384	31.2	5	1.3
	1	0	288	28.8	7	2.4
	0	0	282	28.2	0	0
34	1	19	123	12.3	41	33.0
	1	0	122	12.2	25	20.5
	0	0	219	21.9	3	1.4
40	1	25	388	24.3	235	60.6
	1	0	504	31.5	214	42.5
	0	0	479	24.0	55	11.5

Embryonic Wistar rat fibroblasts were obtained from 14-d-old embryos that were removed under sterile conditions, washed twice in phosphate-buffered saline (PBS) and cut into fragments which were stirred for 5 min in PBS and then submitted to the repeated action of 0.25% trypsin at 37° C. The supernatant from the first 20 min digestion was discarded, and those from the next three digestions, each of 10 min, were combined, filtered through sterile gauze and centrifuged. The pellet was resuspended in Dulbecco's growth medium supplemented with 10% foetal calf serum and this cell suspension was used to prepare primary cultures. After counting and diluting, 10^4 cells were seeded into 10 cm Petri dishes (Falcon Plastics) that contained 10 ml medium, and the dishes were incubated at 37° C in an atmosphere of 10% CO_2. The cells were subsequently subcultured every 5 d. Following the third subculture the Petri dishes were divided into groups and benzo(a)pyrene (Schuchardt) added, as an acetone solution, to the medium in one group of dishes to give a benzo(a)pyrene concentration of 1 $\mu g\ ml^{-1}$, and an acetone concentration of 0.5%. Another group of dishes remained untreated. After 2 h the cells in both groups were subcultured. After a further thirteen passages, half the dishes of treated cells were continuously exposed to phorbol ester (12-O-tetradecanoyl-phorbol-13-acetate, Schuchardt) added as an acetone solution to give a final concentration of 0.01 μg phorbol ester per ml of medium, the ester being added at each medium change. Estimations of plating efficiency and morphological transformation were made at intervals on 200 cells from each group that were cloned in the supplemented growth medium and which, after 9 d, were then fixed in methanol and stained with May-Grunwald and Giemsa.

* Number of passages during which benzo(a)pyrene or phorbol ester were present in the cell medium.

When cells were examined at intervals during the experiments for their ability to form multicellular colonies in semi-solid agar medium, the results given in Table 2 were obtained. These show that although some cells treated with benzo(a)pyrene alone developed the ability to form colonies, normally of only three to five cells, those subsequently grown in medium that contained phorbol ester often developed into dense foci that consisted of more than twenty cells. The untreated cells did not acquire the ability to form colonies in these experiments before the fortieth passage and remained isolated as single cells in suspension in the agar medium.

Although both morphological transformation and colony formation in semisolid medium are accepted as being indicative of malignant transformation in cultured cells, only the induction of tumours in experimental animals by such cells is generally regarded as proof. In the experiments described here, progressively growing tumours regularly developed only in those animals injected with cells that had been initiated with benzo(a)-pyrene and then promoted with phorbol ester (Table 3); these tumours were all palpably detectable 3 weeks after injection of 2×10^6 cells and were transplantable. Cells that had been treated with initiator alone occasionally gave rise to a more slowly growing tumour. On histological examination, the tumours were identified as fibrosarcomas[3,18,19] in which sarcomatous cells in close contact showed a fusiform orientation and atypical nuclei. No tumours have been detected to date in any of the animals injected with untreated cells (Table 3). In other control experiments, uninitiated rat fibroblasts were treated continuously with phorbol ester. Although, after twenty-nine passages in medium containing the promoter, these cells gave rise to a few colonies of up to twenty cells in semisolid agarose medium, this colony forming ability was not apparent before the twenty-fifth passage; when reinjected into animals, cells treated with phorbol ester alone did not give rise to any tumours.

Table 2 Colony Formation in Semisolid Agarose Medium by Cells Treated with Benzo(a)pyrene and Phorbol Ester

	Treatment *		
Passage No.	Initiation with Benzo(a)pyrene	Promotion with phorbol ester	Colony formation in agar medium
29	1	14	+ +
	1	0	+
	0	0	−
34	1	19	+ + +
	1	0	+
	0	0	−
40	1	25	+ + +
	1	0	+ +
	0	0	±

Rat fibroblasts obtained, cultured and treated as described in the legend to Table 1 were examined for colony forming ability in semisolid medium[20]. Cells (1×10^4) were plated in 0.3% agarose medium and incubated at 37° C in an atmosphere of 5% CO_2. After 10 d unstained cultures were scored for colony formation. Symbols used above are −, single cells; +, colonies of three to five cells; + +, numerous colonies of ten to twenty cells; + + +, numerous colonies of thirty to thirty-five cells.

* Number of passages during which benzo(a)pyrene or phorbol ester were present in the cell medium.

Taken together these observations show that malignant transformation of rat embryonic fibroblasts has been obtained in tissue culture by the sequential action of benzo(a)pyrene and phorbol ester. In addition to morphological alterations, this malignant transformation was characterised both by the ability of transformed cells to form colonies in agar medium and by the formation of tumours when such cells were reinjected into animals. Whereas the latter criterion provides the best evidence for malignant transformation, the apparent ability of chemically transformed cells to form colonies in semisolid medium merits further study. Chemically transformed rat fibroblasts resemble hamster kidney cells transformed with polyoma virus in this respect[20] and the use of this technique could offer an alternative quantitative method for assessing malignant transformation *in vitro*. In addition, it may be possible to use the growth of chemically treated cells cloned in agar medium in order to investigate the stage of malignant transformation that has been reached since both the frequency with which colonies form and their dimensions seem to vary with the treatment given[18]. On the other hand, this technique does not enable a study of the morphological aspects of the growth of transformed cells since, in agar medium, they adopt a spherical form.

Table 3 Tumour Formation *in vivo* by Cells Treated *in vitro* with Benzo(a)pyrene and Phorbol Ester

	In vitro treatment *		*In vivo* tumour formation		
Passage No.	Initiation with benzo(a)-pyrene	Promotion with phorbol ester	No. of cells injected $\times 10^6$	No. of animals with tumours	Time for appearance of tumours (weeks)
22	1	7	2	0/13	
	1	0	2	0/10	
	0	0	2	0/10	
28	1	13	2	0/12	
	1	0	2	0/12	
	0	0	2	0/10	
37	1	22	2	10/10	3
	1	0	2	1/10	4
	0	0	2	0/10	
41	1	26	1	11/11	3
	1	0	1	0/11	
	0	0	1	0/11	

Cultured rat fibroblasts ($1\text{–}2 \times 10^6$) that had been treated as described in the legend to Table 1, were injected, as a suspension in culture medium (0.05 ml), subcutaneously into the cranial region of 2-d-old Wistar rats which were subsequently examined at weekly intervals for palpable tumour formation.

* Number of passages during which benzo(a)pyrene or phorbol ester were present in the cell medium.

The two-stage induction of malignant transformation *in vitro* closely parallels the co-carcinogenesis that has been described *in vivo*[13] for, in both situations, a single treatment with benzo(a)pyrene, for example, initiates cells which can subsequently be promoted to overt malignancy by continuous treatment with phorbol ester. The demonstration that co-carcinogenesis can occur in cells in culture may facilitate studies relevant to human disease. It is accepted that a variety of initiating and promoting agents are present in tobacco smoke[21] and in the urban atmosphere[22]. These undoubtedly contribute to the induction of cancer of the respiratory tract in man which probably represents a widespread example of chemical co-carcinogenesis.

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Prostaglandins as Mediators of Reactive Hyperaemia in Kidney

SEVERAL mechanisms have been proposed to account for the reactive hyperaemia which follows arterial occlusion in the kidney, including the intrarenal production of a vasodilator substance[1,2]. Recent work shows that the local generation of a prostaglandin can cause vasodilatation in the kidney[3,4] and can contribute to autoregulation of renal blood flow[5,6]. We have therefore used a prostaglandin synthetase inhibitor, indomethacin[7], to test whether the reactive hyperaemia which follows release of renal artery occlusion is mediated by a prostaglandin mechanism.

Ten mongrel dogs of either sex weighing 9.5–28 kg, were anaesthetised with pentobarbitone sodium (30 mg kg^{-1}) and ventilated with a respiratory pump. Systemic blood pressure was measured with a Statham pressure transducer from a femoral or brachial artery. The left kidney was exposed by transabdominal incision and the renal artery was isolated to a length of 4–5 cm. Care was taken to minimise manipulation of the kidney and of the renal nerves. Renal blood flow was measured with an electromagnetic flowmeter (Biotronix, Model BL-61 0). The ureters were cannulated and urine output was measured with drop counters. Renal blood flow, urine output and systemic blood pressure were recorded continuously on a Beckman Type S-II Dynograph.

The renal artery was occluded with a clamp placed distally to the flowmeter probe. Occlusion was maintained for 3 min and then released. After reproducible effects had been obtained, an intravenous infusion of 0.9% NaCl was given in a dose of about 30 ml kg^{-1}. The fluid volume of the animal was then maintained constant by saline injected from an automatic syringe which replaced each ml of urine excreted with an equivalent amount of saline. Occlusion of the renal artery was then repeated before and after indomethacin (1–5 mg kg^{-1} intravenously). In three experiments sodium content was measured in the urine samples collected in 10–15 min intervals before and after indomethacin administration.

The rate of blood flow through the kidney varied from 100–400 ml min^{-1} in different experiments. The patterns of flow changes after the release of arterial occlusion differed between animals. There was always an initial brief overshoot of flow which was followed either by a gradually reducing phase of increased flow or by a transient decrease before the flow returned to the pre-occlusion value. After saline load, the brief overshoot was higher and the phase of increased flow was potentiated and prolonged. The transient decrease in flow which followed release of occlusion in some experiments was also abolished. Indomethacin administration induced a decrease in resting renal blood flow, urine output and sodium excretion and a reduction of the reactive hyperaemia which followed release of occlusion. The reduction in resting flow ranged from 5% to 24% of the initial value. The average decrease following administration of 1, 2 or 5 mg kg^{-1} of indomethacin was 9%, 13% and 18% respectively. Urine output decreased in all animals and the sodium content of the urine was reduced from 7% to 25% within 1 h after indomethacin administration.

The reactive hyperaemia following release of renal arterial occlusion was decreased or abolished after indomethacin (Fig. 1). Although the initial brief overshoot was not much reduced, the slower phase of increased flow was either reduced (five experiments) or completely abolished (four experiments). In one animal, which interestingly had a raised blood pressure (175/100 mm Hg), indomethacin did not change the reactive hyperaemia.

In the anaesthetised dog, there is a continuous output of a prostaglandin into the renal vein[6,8]. The output is increased by various manoeuvres, including ischaemia[9] and reduction in perfusion pressure[6] and the prostaglandin has been identified as mainly E_2 (ref. 9), which is a potent renal vasodilator.

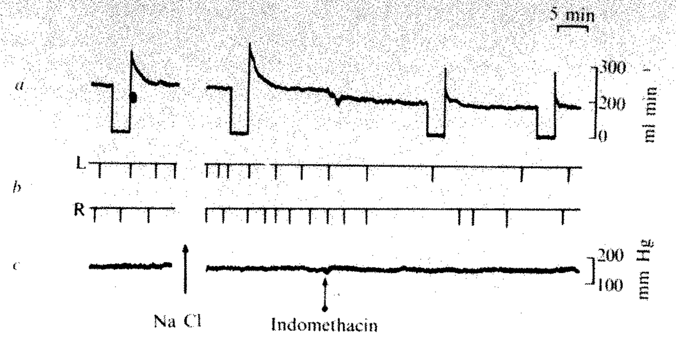

Fig. 1 Effects of intravenous saline and indomethacin on the post-occlusive hyperaemia in the kidney. *a*, Renal blood flow of the left kidney; *b*, urine output (ml) from each kidney; and, *c*, blood pressure of a 9 kg female dog. Each period of occlusion (3 min) was followed by a hyperaemia, the magnitude of which was increased by intravenous saline (30 ml kg^{-1}). After indomethacin (2 mg kg^{-1} intravenously) resting renal blood flow decreased, as did urine flow. All but the fast phase of post-occlusive hyperaemia was abolished.

Indomethacin reduces renal blood flow and abolishes the prostaglandin output[3,6]. Our experiments have now shown that the reactive hyperaemia which follows renal artery occlusion is also reduced or abolished by indomethacin. Thus, the most likely interpretation of our results is that the reactive hyperaemia is at least partially due to a prostaglandin mechanism. It could be that during cessation of blood flow, the basal output of prostaglandin continues within the kidney, or that the ischaemia which is produced actually increases the output. In either situation, the increased concentration of prostaglandin would cause increased vasodilatation, which would express itself by the reactive hyperaemia when blood flow is re-established. As the increased concentration is washed away by the blood, so the vessels would return to their previous tone.

The increase in the reactive hyperaemia which followed saline infusion into the dog also supports this mechanism, for prostaglandins are natriuretic[10] and saline load increases renal prostaglandin output[11]. Thus, an increased prostaglandin production in the kidney may contribute to the increase in urine flow which follows the saline load.

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book reviews

Science of science

Science Observed: Science as a Social and Intellectual Activity. By F. R. Jevons. Pp. 186. (Allen and Unwin: London, August 1973.) £3.75 boards; £2.25 paper.

THE science of science has developed rapidly into a somewhat subsidiary but on the whole almost respectable field of scholarly endeavour. The lack of any comprehensive and generally accepted model of the structure and dynamics of scientific and technological activity, however, cripples much of the work undertaken in this area. It may well be that a "unified field theory" will never really emerge in this particular branch of the social sciences any more than in others. In the meantime, students of science are understandably eager to grasp at the small number of more general hypotheses which have emerged and which seem to bring some element of order into a chaotic universe—often, one is tempted to say, at the expense of reality. Thus, an overview and assessment of progress in the field calls less for synthesis and compilation of a well established body of knowledge than for an explicit and critical examination of the extensive application of a few widely accepted assumptions which may, however, be justified only within very specific and limited conditions.

Professor Jevons has succeeded in providing—in a wonderfully clear, concise and precise manner—with just such an assessment. No doubt one could point to some of the gaps in his presentation, which does not deal directly with the science policy structure that has emerged in recent decades to become an essential component of modern science, or with the controversies on the so-called differences between hard and soft sciences. But *Science Observed* accomplishes its essential objective in putting into perspective some of the basic concepts of science studies in the past decade.

To begin with, the author establishes the new social dimension which calls for more thorough understanding of the scientific universe and the mores of its inhabitants: knowledge is a prerequisite of planning and, however awkward, the planning of science is unavoidable when the waging of war, the limitation of environmental pollution, and the creation of wealth have induced society to enlist the service of a rapidly growing body of researchers. Thus, Weinberg's internal and external criteria are, in many ways, a challenge to provide planners with knowledge of science's internal mechanisms and their relations with society.

The scientific study of science is generally expected to produce objective and quantitative knowledge. Numbers of scientific journals, of abstracts, of papers or of citations are some of the indicators most widely used. But these indicators are not straightforward reflections of quality and progress in science, and are influenced by many extra-scientific features of the research profession and the academic marketplace. Thus, "numerology is interesting but should be used only with the utmost circumspection" since "in particular cases it could be wildly misleading" (page 45). The case would even be strengthened if the author had explored some of the abuses and inaccuracies of research and development statistics.

Unfortunately, as the author points out, the disappointments of quantification are not compensated by reliable qualitative analysis. What is scientific method? Neither the inductive or the hypothetico-deductive theories really account for "inspired guesses" and 'intuitive hunches" which so often seem to underlie scientific creativity. In a historical perspective, T. S. Kuhn's exploration of the structure of scientific revolutions may provide an ideal pattern of evolution of, at least, some disciplines. It remains that his attractive distinction between "normal" and "revolutionary" research is not, in practice, as clear cut as one might wish: "it is difficult not to be convinced by direct inspection of science that contributions form a continuous spectrum" (page 69).

Lacking an acceptable formulation of the laws of the scientific universe, can one at least determine what moves its actors? The striving for recognition by fellow scientists, which has often been presented as the key motivation to scientific work seems only to apply to limited numbers and circumstances, leaving aside such modern developments as teamwork, expansion of research activities in non-academic institutions and increasingly complex career patterns.

The opacity of the internal workings of science is matched by that of the relations between science and society. For example, in spite of many decades of scrutiny, the processes governing the contributions of science to economic growth remain unchartered. Only the complexity of the factors involved become more and more apparent.

Professor Jevon's new book, with its careful exposition and appraisal of the meagre results accumulated to date (an anthology of fundamental writings on the subject is provided in the appendices) should, it is hoped, inspire new, unprejudiced and more ambitious efforts to clarify the complex dynamics of the internal structures and external links of the system of science. It should also serve to caution educators and policy makers against short cuts and rash approximations in dealing with a system that is still not understood. If these results are achieved, the concluding note of optimism on the future of technology may turn out to be not as unrealistic as it may seem today.

GEORGES FERNÉ

High energy physics

Particle-interaction Physics at High Energies. By S. J. Lindenbaum. Pp. xiii+512. (An International Series of Monographs on Physics.) (Clarendon: Oxford; Oxford University: London, October 1973.) £15.00.

THE author of this scholarly work is an internationally recognised experimental physicist and expert, having made his own original contributions at the Brookhaven National Laboratory in this research field on several topics. The book starts at the level of the postgraduate student in high energy physics, and should appeal primarily to research workers in the field. The style is not particularly pedagogic, nor does one set out from first principles. Instead the book traces the development and reviews progress made upon particular topics. The first three chapters deal briefly with introductory matter about pions and meson field theory. The following three chapters contain considerable detail on pion-nucleon scattering, dispersion relations and models of pion production. The next four chapters comprise concise summaries of topics concerning kaon and hyperon physics, particle classifications, and electron scattering. The penultimate chapter is concerned with weak interactions, and the final one is devoted to a substantial review of the field of total cross sections and elastic scattering at very high energies.

From these contents, it is clear that the author has leaned towards the topics with which he has been most directly involved, particularly the physics of hadronic interactions.

The omission of the considerable work done in the last decade to study the hadronic interactions of photons, underlines the dilemma in which any author tackling this subject is placed, since it is impossible to cover all aspects and be up to date at the same time. But the author has taken on, quite successfully, a prodidgous task and can be congratulated on seeing this through to its end. The shorter chapters come over well as concise, yet essentially comprehensive, statements of the facts, while in the main parts of the book, one is left in no doubt as to the author's comprehensive and detailed knowledge of his topics, and of the considerable experimental and theoretical activity in the field over the past twenty years. The reference lists are extensive, and the well-known "Tables of Particle Properties", originating from the "Particle Data Group", round off the volume.

The production standards of the book are of the highest quality, and typographical errors, though present, are rare. Appropriate libraries should be encouraged to purchase the book.

W. GALBRAITH

Serious black holes

Black Holes: Les Houches 1972. Edited by C. DeWitt and B. S. DeWitt. Pp. xii + 552. (Lectures delivered at Summer School of Theoretical Physics of the University of Grenoble.) (Gordon and Breach: New York, London and Paris, 1973.) £13.50.

THIS book has already established itself as an essential work for those interested in black holes. The publication is timely because after some years of theoretical studies evidence is now mounting that black holes may have been observed indirectly in some X-ray sources. Both theoretical and observational aspects are treated in these lectures.

The book begins with Hawking's lectures on the "Event Horizon"—the "one way membrane" which separates the exterior of a black hole from the hidden interior. In them he establishes the general properties of the event horizon using the topological methods which were developed by Penrose, Geroch and himself to prove the singularity theorems which show that according to general relativity the outcome of catastrophic gravitational collapse will be a space time singularity. By assuming that these singularities will lie hidden inside an event horizon ("Cosmic Censorship Hypothesis") he shows that the event horizon must be a null surface any connected component of which may not bifircate and whose area must not decrease with time. He goes on to establish that a stationary black hole must be static or axisymmetric and have spherical topology. Then he applies these ideas to the flow of energy and angular momentum into black holes and to time symmetric black holes.

The flow of energy and angular momentum down a hole is taken up by Carter, who restricts most of his lectures to asymmetric, stationary black holes and gives an account of the thermodynamics of a system of matter and a black hole. Quite apart from its relevance to black holes this is an excellent account of general relativistic thermodynamics. After discussing some important properties of the Kerr solution he goes on to show that the most likely final state of an isolated black hole is that of a Kerr black hole.

Both Carter and Bardeen give a great deal of space to the Kerr metric which must now rank with the Friedman expanding universe metric as the most important exact solutions of Einstein's equations we have. Bardeen provides much detailed material on the geodesics in this geometry and then proceeds to a study of rapidly rotating bodies in general relativity culminating in a fascinating study of self gravitating disks which is of especial importance because they are the only ones so far showing how a rapidly rotating black hole may form.

After reading so much theory the reader is then encouraged to proceed further by considering the data assembled by Gursky on the X-ray observations. In this chapter Gursky indicates some of the observational techniques and their pitfalls and argues that only accretion into compact objects can explain the data. He discusses particular sources in detail—notably Cygnus X1 which may contain a black hole and Hercules X1 which probably contains a neutron star.

From Gursky's lectures it emerges that the most interesting X-ray sources from the point of view of black holes are those in which a bright young star is orbiting an unseen companion in a close binary system. The energy of the X rays is held to arise from the accretion of matter coming from the bright primary star which is spilling over its Roche lobe. Because this matter possesses angular momentum it cannot fall directly in but forms a disc around the companion and angular momentum and energy are transferred outwards by some viscous mechanism. In their lectures Novikov and Thorne treat this transfer of angular momentum with characteristic clarity avoiding the errors of some earlier workers. Because the precise mechanism of viscosity is as yet not understood—turbulence and magnetic fields playing a major role—they provide a rather general analysis of accreting discs leaving plenty of room for viscosity parameters to be plugged in later. They also build some detailed models however, and give a useful summary of the astrophysical principles behind the calculation of the various emission processes.

In the final set of lectures Ruffini discusses a variety of important topics amongst which stand out an attempt to determine the maximum possible mass of a neutron star on rather general physical grounds and an account of why such a maximum exists at all. This is obviously of especial importance when discussing actual binary systems. He also gives a number of calculations of the gravitational waves emitted by particles falling down a hole, though some of the calculations on charged black holes should be treated with care because the coupling between electromagnetic and gravitational perturbations seems to have been ignored. This section contains much of interest but the format (short introductions followed by photo-reproductions of reprints and preprints) does not make for easy reading or compact presentations.

To finish: this is an important book which everyone seriously interested in black holes should read; I hope for the sake of their pockets that a paperback edition will be produced soon.

G. W. GIBBONS

Methods for metals

Physical Metallurgy: Techniques and Applications. By K. W. Andrews. Vol. 1 pp. 349; vol. 2 pp. 347. (Allen and Unwin: Hemel Hempstead, August 1973.) Vol. 1 £7.95; vol. 2 £8.20.

BOOKS of this type are usually compilations by various specialists whose contributions are under the control, to a greater or lesser degree, of an editor. The present book has the great advantage of having been written by one author of wide experience who has brought to the task an impressive expertise and has imposed on the book a most desirable uniformity of treatment. It could be argued that several of the topics, such as electron microscopy and crystallography, have been adequately dealt with in specialist books, but there is much to be said for a concise summary of the principles of a technique together with some carefully selected examples of its use. Dr Andrews is not content simply to describe a technique, but goes to considerable lengths to provide compact quantitative treatments of the various physical principles involved, in a way which will enlighten the serious student, and refresh the memories of established scientists who may have long taken the underlying principles for granted.

The first volume is rather dominated by the subject of X-ray and electron diffraction and associated techniques, which together with an introductory outline of crystallographic principles takes up two thirds of the volume. This perhaps reflects the author's own research interests, but it is appropriate that methods which have been so effective in physical metallurgy should receive substantial emphasis. It does however mean that more recently developed techniques such as field emission and field ion microscopy receive much less attention than they deserve. Other subjects dealt with in this volume include thermal methods and solidification techniques. The former deserve a place in any book of this type, for although they reach back almost to the genesis of the subject, in their modern forms they are still extremely valuable methods of investigation. The chapter on solidification techniques is rather brief and apart from a useful treatment on zone refining does little to stress the importance of this stage in alloy preparation.

The first part of Volume 2 deals with both optical and electron microscopy. It is refreshing to see that the rapidly growing subject of quantitative metallography receives due attention, while there is a fifty-page review of electron microscopy which provides clear simple treatments of image formation and the basic contrast effects. The latter half of this volume is likely to be particularly useful, as it deals with several topics about which rather less has been written recently. In particular the chapter on the determination of internal stresses is an excellent review which again reflects a special interest of the author, who has taken pains to spell out the underlying principles of stresses on macro and micro scales, and also around dislocations. The other chapters in this volume are concerned with electrical and magnetic methods, and with internal friction techniques. The latter subject has not received much attention recently, so a compact review of this type is likely to prove most valuable.

In summary these two volumes provide a most useful survey of a number of physical techniques important in metallurgical research. The able combination of basic theory with practical application of the techniques should appeal to a wide spectrum of readers.

R. W. K. HONEYCOMBE

Phoenix palaeontology

Evolutionary Paleoecology of the Marine Biosphere. By J. W. Valentine. Pp. xv + 511. (Prentice-Hall: Englewood Cliffs, NJ, October 1973.) $6.95.

THE past few years have witnessed striking advances in the application of modern concepts of ecology, evolutionary biology and plate tectonics to achieve an improved understanding of the fossil record, and it is appropriate that the first comprehensive review of this revitalised palaeontology should be written by one of the leading workers in this sphere. In the early part of his book Valentine gives a useful survey of evolutionary and ecological theory and of the principal physical parameters of the marine environment. He goes on in the more substantial part of the book to treat marine animals in terms of a hierarchical system. Successive chapters deal with the mode of life and functional morphology of individual organisms, populations, communities, faunal provinces and the marine biosphere, ending with general interpretation of the evolutionary history of this biosphere from the Precambrian to the present. Each level in the hierarchy can be viewed as a sort of 'black box', with its own intrinsic patterns of organisation which can often be studied without reference to lower or higher levels. Thus when dealing with patterns of community organisation one does not necessarily have to be preoccupied unduly with the relationship of form and function for particular phenotypes, and so on.

One of the most impressive features of this fine book is the overall conceptual framework created, which breaks away refreshingly from the somewhat stultifying preoccupation with particular taxa, with no particular scientific goal in mind, which has characterised so much palaeontology in the past. Another is the erection of a series of testable models. It is not that detail is ignored, far from it, but it is subsumed into a more general system so that one can evaluate more readily the relevance of making certain types of observation as a means of testing particular hypotheses.

Valentine's own research has been mainly concerned with the higher organisational levels and his important views on such matters as the environmental control on provinciality, diversity changes, mass extinctions and the evolution of basic morphological structures through geological time are naturally given a good airing. The presentation of these views is, however, not dogmatic and one does not have to agree with all he says to accept that he is laying emphasis on the sorts of problems to which the palaeontological community should address itself increasingly in the future. The writing style is generally clear and where new jargon is introduced it is well defined. The production is generally good, and illustrations abundant, though the number of typographical errors is large. The book can be used with profit in advanced undergraduate courses and deserves to be read by all who want to learn more about the new palaeontology.

A. HALLAM

Biological names

Biological Nomenclature. By Charles Jeffrey. Pp. ix+69. (Arnold: London, October, 1973.) £2 boards; £1 paper.

"WHAT! Is it possible? Not to know the principles of nomenclature? Of what can your teachers have been thinking?"

Older biologists should no longer need thus to paraphrase Rob Roy. Dr Jeffrey's book shows clearly how far there is a simple common basis to the various international codes of biological nomenclature, and how they differ in expression.

Dr Jeffrey is a botanist. A zoologist may find points to criticise, of which I mention two. First, it is not true that publication is the process of making a zoological name available (page 64) (see chapter 4 of the zoological code). Second, the notion of the "nominal taxon" is misrepresented. It is not enough to say (page 63) that it is the taxon that bears a given name: it is the taxon, as objectively defined by its type, to which a given name applies. Botanists speak of "the type of a name", which is to imply, in cases of homonymy, that one concept can have two or more different objective bases, which is a nonsense. The name is not the same thing as the concept it denotes. To Dr Jeffrey "The type of a name . . . has no significance for classification" (page 18). To a zoologist, the nominal taxon is the concept that is common to all notions of the taxon. As such, it provides the essential link between classification and nomenclature and is a notion of great practical value.

Nevertheless, biologists at all levels of experience should study this book. Dr Jeffrey and the Systematics Association have rendered a major service to all biologists whose work calls for some understanding of the basic principles of nomenclature.

R. V. MELVILLE

obituary

D. M. S. Watson

PROFESSOR D. M. S. WATSON, FRS, who died on July 23, aged 87, was trained at the University of Manchester as a chemist and geologist, but started research in palaeontology. His first palaeobotanical publication appeared when he was twenty and the second (jointly with Marie C. Stopes) was published by the Royal Society only a year later. His early papers include descriptions of a fossil king-crab and the loops of a brachiopod, and within five years of entering vertebrate palaeontology he had published on fossil fish, amphibians, rhynchosaurs, plesiosaurs, crocodiles, chelonians and mammal-like reptiles.

He developed many of these interests in later years, and in particular began to ask two questions: how did it work and why did it change? By so doing he joined Abel as one of the pioneers of palaeobiological thought, and became a leader in his field by the age of 30. Much of Watson's work was based on his own collecting in South Africa and he wrote several papers on Karroo stratigraphy. He was soon recognised as an authority on the mammal-like reptiles, and he began to build up his experience of labyrinthodont amphibians and eotylosaurs, while exploring (jointly with Hankin) the flight of pterodactyls.

Watson was at his greatest in the period between the two World Wars, and his papers on the structure, evolution and origin of the Amphibia stand out as classics. He was appointed to the Jodrell Chair of Zoology and Comparative Anatomy at University College, London in 1921, elected Fellow of the Royal Society in 1922, gave their Croonian Lecture in 1924 and the Romanes Lecture in 1928.

D. M. S. was particularly interested in missing links in the evolutionary record because they held promise of an opportunity to study the functional aspects of transition from one mode of life to another. His quest to fill the structural gap between jawless and jawed fish led to his recognition of the aphetohyoid grade in acanthodians and, although his interpretation has been doubted, his thorough study of this difficult material remains a masterpiece. Other topics which he explored include the evolution of the mammalian ear, the primitive tetrapod limb, the structure of the monotreme skull and fossil coelacanths. For many years Watson was aided in his work by Miss Joyce Townend whose excellent illustrations grace his publications.

By present standards Watson's mechanical preparation of fossils was relatively crude, but his training in geology ensured an ability to visualise three dimensional structure from a series of broken surfaces, and he could break a rock in just the right place to confirm his expectations. He was not a writer of textbooks and communicated best through his lectures, during which he radiated enthusiasm. The spirit of these is captured in *Paleontology and Modern Biology*, which contains the Silliman Memorial Lectures, essentially as they were delivered at Yale in 1937. Another fine performance was his lecture on *Pterodactyls*, which he gave on several occasions but never published.

On the personal side, D. M. S. sometimes seemed slightly reserved (he had a charmingly gentle relationship with his wife) but he always found time to listen to his research students and he was tolerant of their inexperience.

Announcements

Appointments

Major-General Sir Leonard Atkinson, KBE, has been elected the new chairman of the Council of Engineering Institutions.

Awards

The 1973 International Health Foundation Award for papers on *Diagnostic X-ray exposure and the human embryo* will go to Dr Willem Herstel of the Department of Radiology, University Hospital, Leiden.

Errata

IN the article 'Alteration in Heterocyst Pattern of *Anabaena* produced by 7-Azatryptophan' by G. J. Mitchison and Michael Wilcox (*Nature new Biol.*, **246**, 229; 1973) the diagram captioned 7-azatryptophan shows tryptophan and not the 7-aza derivative. The nitrogen was inadvertently replaced by a carbon during preparation for the printer.

In the article 'Formation of Lunar Carbide from Lunar Iron Silicates' by C. T. Pillinger *et al.* (*Nature phys. Sci.*, **245**, 3; 1973) Figure 1 legend, lines 4 and 5 should read "^{36}Ar data from Heymann and coworkers$^{5-8}$ (□) and Bogard and coworkers$^{9-11}$ (○): . .". Paragraph 7, line 9 should read "at least 10^2 times that necessary for the reaction. Conversely, . .".

IN the article 'Tetrodotoxin and Cobalt Blockade of Neuroblastoma Action Potentials' by Ilan Spectro, Yosef Kimhi and Phillip G. Nelson (*Nature new Biol.*, **246**, 124; 1973) the first line should read 'The mouse C-1300' not 'C-13000'; paragraph 4, line 7 should read 'second, slowly rising peak . . .'; Table 1 legend, line 1 should read $MgCl_2 = 1$; $CaCl_2 = 2$ (not $MgCl_2 = 2$); ref. 5 was published in 1971 not 1972, and the last author in ref. 8 is Nirenberg, M., not Ninberg, M.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

The Natural Environment Research Council. Publications Series B, No. 7: Research in the Geological Sciences. (A consultative document comprising reports from eleven NERC Geological Sciences Working Parties.) Pp. ii+113. (London: The Natural Environment Research Council, 1973.) [111

The Zoological Record. 1970, Vol. 107, Section 16: Amphibia. Compiled by the Staff of the Zoological Society of London. Pp. vi+79. £6.65. 1970, Vol. 107, Section 17: Reptilia. Compiled by the Staff of the Zoological Society of London. Pp. 90. £6.65. (London: The Zoological Society of London, 1973.) [211

Scientific Proceedings of the Royal Dublin Society. Series A. Vol. 4, No. 31: Lower Palaeozoic Graptolitic Facies in Ireland and Scotland—Review, Correlation, and Palaeogeography. By J. A. Weir. Pp. 439–460. 75p. Vol. 4, No. 32: Variolitic Lavas from South County Wicklow, Ireland. By J. C. Brindley and S. Millan. Pp. 461–469. 75p. Series B. Vol. 3, No. 13: The Medals of the Royal Dublin Society. By Arthur E. J. West. Pp. 165–190+plates 1–7. £1. Vol. 3, No. 14: Observations on the Swarming Behaviour of the Polychaete *Platynereis dumerilii* on the West Coast of Ireland. By John P. Mercer and J. Dunne. Pp. 191–194+plate 10. 30p. Vol. 3, No. 15: The Natural Mortality of Cabbage Root Fly Eggs in Peatland. By M. F. Ryan and J. Ryan. Pp. 195–199. 20p. (Dublin: Royal Dublin Society, 1973.) [211

National Physical Laboratory. Corrosion of Metals Research 1924–1968. By Frank Wormwell. Pp. xix+73. (London: HMSO, 1973.) £1.65 net. [511

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G. G. Meynell

This book first shows how different types of plasmid were noticed and then goes on to the physical structure of their chromosomes, their manner of replication and how they act to bring about conjugation and other processes they determine. Although the treatment is primarily biological, the practical scope of the subject, as regards both laboratory techniques and the implications for human and animal medicine, have been kept in mind by including appropriate references.

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L. A. J. Venverloo

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British Journal of Radiology

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D.E.S. Truman B.A. PH.D. February 1974. 144 pages, 53 illustrations. Cloth £3.50, paper £2.00

Insect Population Ecology: an analytical approach
G.C. Varley, G.R. Gradwell and M.P. Hassell. 1974. 232 pages, 73 illustrations. Paper, £2.75

Practical Genetics
Edited by P.M. Sheppard. M.A. D.PHIL. F.R.S. 1974. 344 pages, 28 illustrations. £8.50

The Structure of Marine Ecosystems
John H. Steele. April 1974. 128 pages, 30 illustrations. Paper, £2.75

Chemical Analysis of Ecological Materials
Edited by Stewart Allen, Max Grimshaw, John Parkinson and Christopher Quarmby. June 1974. 352 pages, 60 illustrations. About £8.00

The Biology of Brains
Edited by W.B. Broughton. May 1974. 336 pages, 40 illustrations. About £6.00

Algal Physiology and Biochemistry
Botanical Monographs Volume 10, edited by W.E.P. Stewart. April 1974. 960 pages, 75 illustrations. £15.50

Biology in Pest and Disease Control
Thirteenth Symposium of the British Ecological Society, edited by D. Price Jones M.SC. PH.D. F.I.BIOL. and M.E. Solomon M.SC. F.I.BIOL. April 1974. 272 pages, 23 illustrations. £6.50

Quaternary Plant Ecology
Fourteenth Symposium of the British Ecological Society, edited by H.J.B. Birks and R.G. West. 1974. 336 pages (oblong), 144 illustrations. £13.00

Cell Wall Deficient Forms
Lida H. Mattman PH.D. 1973. 320 pages. CRC Press, £15.00

Diffuse Reflectance Spectroscopy in Environmental Problem-Solving
R.W. Frei PH.D. and J.D. MacNeil PH.D. February 1974. 324 pages. CRC Press, £20.50

Mass Spectrometry of Pesticides and Pollutants
S. Safe and O. Hutzinger. February 1974. 240 pages. CRC Press, £16.75

Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

Drug Receptors
Edited by H. P. Rang

The 1972 Biological Symposium on Drug Action brought together experts from all over the world to discuss the separate and varying approaches that are being used to study drug receptors. This book contains the proceedings of that symposium, which includes a discussion of the rapidly developing field of the labelling of cholinergic receptors and the application to drug receptor mechanisms of ideas that have been developed in relation to enzyme regulation. £7.80

Biological Effects of Drugs in Relation to their Plasma Concentrations
Edited by Donald S. Davies and B. N. C. Pritchard

In considering the relationship between the biological effects of drugs and their plasma concentrations this indispensable work brings together for the first time a large proportion of the available data on this subject. Discussion of whether the monitoring of drug levels in plasma should be part of clinical practice is also included. £6.50

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Edited by James E. Prier and Herman Friedman

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The Zoological Society of London

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Applications are invited for the post of DIRECTOR of the WELLCOME INSTITUTE OF COMPARATIVE PHYSIOLOGY.

The Institute was established in 1962 and since then has been engaged on various aspects of reproductive physiology. The staff consists of three post-graduate workers, three students and eleven supporting staff. The Institute is financed by the Zoological Society of London with the help of funds from the Ford Foundation and other grant-giving organisations.

The Director will be required to provide scientific leadership of high calibre and actively participate in research, administer the Institute and advise the Society, when required, on breeding problems. The Director should have the ability and drive to keep the Institute in the forefront of research.

Candidates should be Biologists with considerable experience in the field of reproductive physiology and with experience of administration.

The post will become vacant in September 1974 and carries a salary related to the Professional range of London University with F.S.S.U. benefits. Applicants should send six copies of a full curriculum vitae and the names of three referees to:

The Establishment Officer,
The Zoological Society of London,
Regent's Park, London NW1 4RY.

Closing date 31st March, 1974

(584)

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DEPARTMENT OF PHYSICS

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Letters of application (two copies), including curriculum vitae and the names of two referees, should be sent to the Establishment Officer, University House, Lancaster LA1 4YW, by March 31, 1974. (702)

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☐ Application forms (for return by 5 March 1974) from Scottish Office, Personnel Division, Room 214, 22/25 Queen Street, Edinburgh, EH2 1JX, telephone 031-556 9222.

Department of Agriculture and Fisheries for Scotland
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Department of Trade and Industry
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☐ Application forms (for return by 5 March 1974) from WSL, P.O. Box 20 Gunnels Wood Road, Stevenage, Herts, SG1 2BX.

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☐ Application forms (for return by 1 March 1974) from Assistant Director, MRE, Porton Down, Salisbury, Wilts.

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(676)

Clinical Chemists

The Searle Group is a leading research-orientated company in the pharmaceutical industry. Scientists are required to join an expanding department concerned with the development of diagnostic products, reagents and methods. Applicants should have qualifications such as BSc or FIMLT in Biochemistry, Clinical Chemistry or a related science, but a good scientific attitude is as important as the precise field of study. Experience of general clinical chemistry, enzymology, immunology or radioimmunoassay is desirable for some of these posts, which should appeal to those interested in seeing practical use made of their work. Salaries are attractive and negotiable and Company benefits include: a contributory Pension Fund, BUPA membership scheme and 4 weeks holiday.

Please apply in writing giving full details of career and education to: J N Williams
Assistant Personnel Manager,
Searle Diagnostic, G D Searle & Co Ltd,
Lane End Road
High Wycombe
Bucks

SEARLE

(667)

UNIVERSITY OF EDINBURGH

Chair of Forestry and Natural Resources

The University Court invites applications for the above Chair. The Department, of which the Professor is the Head, is in the Faculty of Science and is one of twelve departments in the School of Biology. At present it teaches for the degree of B.Sc. (Ecological Science) with four Honours Schools in Ecology, Forestry, Resource Management and Wildlife and Fisheries Management. The problem of utilisation of natural renewable resources is of particular importance in the Scottish context, and the new Professor will be expected to continue to forge links and promote developments in this area.

The salary will be within the professorial range and not less than £6,885, together with superannuation under FSSU.

The terms and conditions of appointment together with further particulars may be obtained from the undersigned, with whom applications (twelve copies), giving the names of three referees, should be lodged not later than February 28, 1974. A candidate from overseas may submit one copy of his application. Applications to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL. Please quote reference number 1/74. (666)

SK&F

Research Organic Chemist

We have a vacancy for an organic chemist to join our medicinal chemists in synthesising compounds of interest as potential drugs. The work is in a stimulating environment on a successful research programme.

We require a skilful practical chemist up to first degree level (G.R.I.C., B.Sc., H.N.C. or equivalent qualifications), preferably under 26 years old. Experience of organic synthesis is desirable.

We offer a competitive salary related to age, qualifications, experience and performance on a progressive scale.

Other benefits include contributory pension and life assurance scheme, annual bonus, subsidised cafeteria and flexible working hours.

Our modern Research Institute, part of the European Research function of a major international pharmaceutical organisation is situated in Welwyn Garden City within half an hour of Kings Cross main line station.

Please write, giving brief relevant details of age, qualifications, and experience to:

The Personnel Officer,
Smith Kline & French Laboratories Limited,
Mundells, Welwyn Garden City, Herts.

(675)

A member of the worldwide **SmithKline** Corporation

Erasmus University Rotterdam

Announcement of a vacancy to the trade group Pathology (control department Morbid Anatomy II) at the faculty of medicine.

Reader in Experimental Pathology

The functionary will give a scientific and organizing guidance to the research group of the trade group, supervise promovendi as a promoter, prepare and supervise optional practical work of third-year medical students.

They, who possess a large experience in either the immunopathology, or the molecular biology (particularly on tumour virology) are preferred.

Also people who possess experience in these areas, but are not on the registers of morbid anatomy, are considered.

They, who want to be considered for the function as well as they who want to draw attention to possible candidates, can write to the Secretary of the Faculty, Dr. Masurel, Postbus 1738, Rotterdam.

You can apply for information to the head of the department Morbid Anatomy II, Prof. Dr. M. J. de Vries, Postbus 1738, Rotterdam, Telef. 361000, extension 2703.

(658)

Beecham Pharmaceuticals

Fermentation Development

Microbial Physiologist

A microbial physiologist is required in the culture improvement section of the fermentation development group. The work will involve investigations into the novel characteristics of improved penicillin producing cultures, liaising closely with the culture improvement team.

A background of fungal physiology, allied to an interest in genetics, is desirable.

Microbial Biochemist

A biochemist with an interest in the biosynthesis of secondary metabolites is required for investigations into the metabolic control of penicillin production. Excellent facilities exist for studying laboratory, pilot plant and large scale production fermentations.

Ideally, applicants for this position should have a higher degree or at least three years' relevant post-graduate experience.

We offer competitive salaries and attractive fringe benefits, including a non-contributory pension and life assurance scheme. Please write giving brief details of age, qualifications and experience to: Miss C. Turnbull, Personnel Officer, Beecham Pharmaceuticals, Clarendon Road, Worthing, Sussex.

THE QUEENS AWARD TO INDUSTRY 1966 1970 1971 1972

(642)

BP

Microbiologists

BP Research Centre, Sunbury

We are looking for graduate microbiologists who are interested in a career in the development of new processes for conversion of petroleum based substrates into commercially important fermentation products. They will join a team involved in the isolation, genetic modification and evaluation of the process performance of micro-organisms.

Senior Microbiologist

preferably Ph.D. with several years' post-doctoral experience. He should have a special interest in cell physiology, biochemistry and genetics. Age range 26–35.

Microbiologist

Should have a good honours degree plus an interest in culture enrichment techniques and culture stability in continuous fermentations. Age range 23-27.

■ Please write, giving brief details of age, qualifications and experience and quoting reference U.709, to: The Manager, Central Recruitment, The British Petroleum Company Limited, Britannic House, Moor Lane, London EC2Y 9BU.

(593)

Kendal Morecambe Lancaster

North Lancashire & South Westmorland Hospital Management Committee

SENIOR GRADE BIOCHEMIST

in the Group Laboratory at the Royal Lancaster Infirmary

Duties will include the supervision of routine work and quality control, the development of new techniques and certain more specialised procedures.

The Laboratory is well supplied with automated equipment and an extension has recently been built to accommodate this section.

The person appointed should have wide experience in automated techniques and an interest in computers would be an advantage.

The department (Tel: 0524 2345) may be visited by arrangement with Dr. Peter Harvey, Consultant Pathologist or Mr. Allan Bowers, Principal Biochemist.

Applications including the names of two referees should be sent to
The Group Secretary,
North Lancashire and South Westmorland Hospital Management Committee, Lancaster Moor Hospital,
LANCASTER,
to arrive before 28th February, 1974.

(AK691)

THE QUEEN'S UNIVERSITY OF BELFAST

LECTURESHIP IN MICROBIOLOGY

Applications are invited for a Lectureship in the Department of Microbiology and Immunobiology from April 1, 1974, or such other date as may be arranged. The successful applicant should have had experience in clinical microbiology and the immunology of infectious agents. Research experience in those fields would be a distinct advantage. The duties of the appointment include teaching to science and medical students. The salary will be in the range £2,553-£4,548. If medically qualified, the successful applicant may be paid an additional salary on a sessional basis by the Eastern Health and Social Services Board for hospital services undertaken over and above University duties. Both salaries carry contributory pension rights under the FSSU. The initial placing will depend on qualifications and experience.

Applications should be received by March 5, 1974. Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast BT7 1NN. (Please quote Ref. 74/N.) (591)

UNIVERSITY OF NAIROBI

KENYA

Applications are invited for the post of PROFESSOR IN DEPARTMENT OF ANIMAL PRODUCTION. Applicants, who must have a Ph.D. in either Animal Nutrition, Genetics and Breeding or Ecology and Management, should also possess a good general background in the other branches of Animal Production. They must have done several years of relevant university teaching and have experience in directing postgraduate students. A knowledge of animal production systems in the tropics, preferably in East Africa is essential. Appointee will be required to teach both Veterinary and Agriculture students and to conduct Animal Production practicals in addition to doing research within his field of specialisation. Salary scales: K£3,600-K£4,380 p.a. (K£1=£1,29 sterling.) The British Government may supplement salary by £1,250 p.a. (sterling) for married appointee or £950 p.a. (sterling) for single appointee (normally free of all tax) and provide children's education allowances and holiday visit passages. FSSU; Family passages, various allowances.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be forwarded by air mail, not later than March 12, 1974, to the Registrar, University of Nairobi, P.O. Box 30197, Nairobi, Kenya. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars of these appointments are obtainable from either address. (608)

UNIVERSITY OF KHARTOUM

SUDAN

Applications are invited for the following posts in the Faculty of Medicine:

1. SENIOR LECTURER/LECTURER IN BIOCHEMISTRY.
2. SENIOR LECTURER/LECTURER IN PHYSIOLOGY. Teaching experience in Physiology for Medical and Dental students is desirable.

Salary scales: Senior Lecturer £S2,400-£S2,800 p.a. Lecturer £S1,500-£S2,300 p.a. (£1 sterling=£S0.82.) The British Government may supplement salaries in range £1,300-£1,600 p.a. (sterling) for married appointees or £800-£900 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages; various alowances; superannuation scheme; annual overseas leave.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than March 6, 1974, to the Personnel Secretary, University of Khartoum, P.O. Box 321, Khartoum, Sudan. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (609)

UNIVERSITY OF LONDON

INSTITUTE OF PSYCHIATRY

DEPARTMENT OF NEUROLOGY

RESEARCH WORKER required immediately for biochemical/physiological studies in experimental epilepsy. Neurochemical experience an advantage. Applicants wishing to visit the laboratory may phone 01-703 5411, ext. 370, for an appointment. Initial salary up to £1,929 plus £162 London allowance, renewable annually up to 3 years.

Apply to the Secretary, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, quoting reference BSM/N. (652)

QUEEN MARY COLLEGE

University of London

POSTDOCTORAL POSTS IN MILLIMETRE-WAVE AND INFRARED ASTRONOMY

Physicists with experience in astronomy or electronics are invited to write for details of postdoctoral posts in the Physics Department for research in association with any of the following studies:

Radioastronomical (heterodyne) mapping at 1.3 and 2.6 mm.
Submillimetre-wave radiometry of point sources.
Determination of the submillimetre cosmic background.

The first two currently involves use of the Tenerife and Kitt Peak ground-based telescopes and the third uses balloon-borne platforms. The posts could, where appropriate, include some undergraduate or postgraduate teaching.

Enquiries (including brief outline of experience and interests), to Professor J. A. Bastin, (N) Queen Mary College, Mile End Road, London E1 4NS.

(605)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)

Medical Microbiology

Advanced training in bacteriology, virology, mycology, microbial genetics and immunology as applied to medicine, preventive and community medicine, is provided through a full-time course of study for the University of London

Diploma in Bacteriology

This course is open to selected graduates in Medicine, Dentistry, Veterinary Medicine, Science or Pharmacy of this or other approved universities.

Facilities for research leading to Ph.D or M.D. may be offered to suitable students after successful completion of the Dip. Bact. (Lond.).

Closing date for applications: 15th April, 1974.

Further particulars may be obtained from The Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT.

(604)

THE QUEEN'S UNIVERSITY OF BELFAST

DEPARTMENT OF PURE AND APPLIED PHYSICS

RESEARCH ASSOCIATE IN ASTROPHYSICS

Applications are invited for the post of Research Associate tenable from October 1, 1974. The appointee will carry out research (experimental or theoretical) in the astrophysics section of the space and atmospheric physics research group, whose current programme includes high resolution spectroscopic studies of stars and of the interstellar gas using instruments developed for high altitude balloons. Ground-based and satellite observations are planned. Candidates should possess a Ph.D. degree and have some relevant experience in spectroscopy, optics and astrophysics. Salary will be dependent on qualifications and experience and within the range £1,929-£2,223, normally plus F.S.S.U. Appointment will initially be for one year, but is renewable annually thereafter.

Applications, giving full details of academic record and experience, together with the names of two referees, should be sent to the Personnel Department, The Queen's University of Belfast, Belfast BT7 1NN, N. Ireland, before March 15, 1974. (641)

AGRICULTURAL RESEARCH COUNCIL

LETCOMBE LABORATORY

An AGRONOMIST is required to carry out a series of experiments to investigate how soil conditions for crop growth are affected when direct drilling has been practised for an extended period. Duties will include taking day to day charge of all field work.

Applicants should have a degree (preferably honours) in agriculture or an agricultural science. Post graduate experience desirable.

The appointment will be in the grade of Scientific Officer (£1,590-£2,177) or Higher Scientific Officer (£2,076-£2,667). At least five years post graduate experience are required for appointment at the higher grade. The post is superannuated with a non-pensionable allowance to offset personal contributions.

Application forms and further particulars may be obtained from the Secretary, Letcombe Laboratory, Wantage, Berkshire. The closing date for application is February 28, 1974. (599)

UNIVERSITY OF WESTERN ONTARIO

Applications are invited for the position of ASSISTANT or ASSOCIATE PROFESSOR. Responsibilities involve teaching undergraduate and graduate students and conducting a research programme in biochemistry. Ph.D. and research experience in fields of physical biochemistry, membrane biology, or related areas will be given preference.

Send curriculum vitae and three references to Dr. W. C. McMurray, Secretary, Selection Committee, Department of Biochemistry, University of Western Ontario, London, Ontario, Canada N6A 3K7. (261)

COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

Advertisement No. 21/73

It is proposed to appoint a

DIRECTOR

to head the Central Drug Research Institute, Lucknow

Job Requirements:

It is a top research management post in the area of drug research and development. The Director is required to provide high level leadership in the planning and execution of R & D programmes and projects, organising and coordinating multi-disciplinary team work in various aspects of drug research and development.

The scope and functions of the Institute are: utilisation of plant, animal and microbial resources for development of drugs and pharmaceuticals; development of indigenous technology for drugs and drug intermediates, fermentation chemicals; development of new therapeutics and contraceptives and the related basic oriented studies.

He will have the overall responsibility for the functioning of the Institute and for creating the necessary atmosphere conducive to R & D. He should be able to correlate the results of research and development and assure their application and utilisation in industry. The Director is expected to be a research guide, coupled with aptitude for scientific and industrial R & D management.

Qualifications/Experience:

There is no standard application form prescribed as such. The candidate must have high academic attainments, scientific standing and R & D experience in an area of science, technology or industry related to Drugs and Pharmaceuticals. He should furnish proof of independent R & D work and of ability to guide, plan and direct programmes in various aspects of drug research and development, consistent with the job requirements specified above.

Salary/Conditions of Service:

There are five salary scales ranging from Rs. 1600/- to Rs. 3,000/- for the post of Directors in the National Laboratories/Institutes of the Council of Scientific & Industrial Research. Persons selected may be fixed in any one of these scales according to individual merit.

The person selected will be appointed on contract for a period of six years, subject to confirmation of the contract after two years of satisfactory service. Other conditions of contract will be supplied on request.

Age Limit: Below 50 years, relaxable in special cases.

All correspondence in this regard may be addressed to the Chief (Administration), Council of Scientific and Industrial Research, Rafi Marg, New Delhi-1 and the completed curriculum vitae proforma must be received in this office on or before 14.3.74.

(685)

Glaxo

Use your scientific qualifications in Product Registration

Our Product Registration Department based at Greenford is primarily responsible for the preparation, submission and progressing of applications for product licences and clinical trial/animal test certificates, for human and veterinary medicinal products, and for the subsequent review and amendment of these licences and certificates to meet statutory requirements. Other responsibilities include—advising other departments on the procedures to be adapted to ensure compliance with the Medicines Act and subsequent orders and regulations—providing information for group head-quarters to assist with registration of products overseas —acting as the focal point for liaison with the Licensing Authority on queries relating to licences and test certificates.

The work involves liaison with technical and commercial departments throughout the company and provides an overall interest in all aspects of new product development. Contact with colleagues of various scientific disciplines is necessary.

Candidates should have a broadly based scientific qualification, e.g., in pharmacy and a knowledge of and preferably experience in some aspect of new drug development. A more specialised scientific qualification together with broader experience in new drug development might be suitable.

The company offers excellent conditions of employment, including pension and profit-sharing bonus schemes.

Please write giving brief details and quoting ref. U.711
to the Personnel Officer (JSP),
GLAXO LABORATORIES LTD., Greenford, Middlesex

(653)

UNIVERSITY OF LANCASTER

DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited from post-doctoral or graduate candidates in zoology, physiology or biological sciences for an appointment of RESEARCH ASSISTANT or ASSOCIATE tenable from April 1, 1974, or earlier by arrangement for a period of one year to investigate the Metabolism of Electrolytes in Fishes under the direction of Professor W. T. W. Potts. Salary will be Assistant: £1,404 plus FSSU, Associate: £1,929 plus FSSU.

Further details are available from the Administrative Officer, Department of Biological Sciences, University of Lancaster, Lancaster LA1 4YQ, to whom applications should be forwarded not later than February 28, 1974. (677)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF CANCER STUDIES

RESEARCH FELLOW

Applications are invited from biochemists with an interest in tumour virology to join a group working on the detection of virus nucleic acids. The fellowship is supported by the Cancer Research Campaign. Candidates should be post-doctoral and have experience in nucleic acid chemistry. Salary for a Fellow is on the scale £1,929 to £2,223 but a higher point on the Lecturer scale would be considered for a well qualified candidate.

Applications with curriculum vitae and names of two referees to Professor D. G. Harnden, Department of Cancer Studies, Medical School, Birmingham B15 2TJ, by March 11, 1974. (529)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF CANCER STUDIES

RESEARCH ASSOCIATE

Applications are invited for a Research Associate to work on a study of virus expression in somatic cell hybrids. This study is part of a project examining tumour virus persistence and function, and is supported by the Cancer Research Campaign. Candidates should have a good degree in a biological science with experience in tissue culture and/or genetics. Salary will be on the scale £1,569 to £2,223.

Applications with curriculum vitae and names of two referees to Professor D. G. Harnden, Department of Cancer Studies, Medical School, Birmingham B15 2TJ, by March 11, 1974. (530)

UNIVERSITY OF MANCHESTER

LECTURER IN BIOCHEMISTRY

Applications invited for this post, to assist with the teaching of Biochemistry to Honours and subsidiary students in the Department of Biochemistry in the Faculty of Science. Salary range per annum: £1,929 to £4,548; initial salary point not above £2,388. F.S.S.U.

Particulars and application forms (returnable by March 9) from the Registrar, The University, Manchester M13 9PL. Quote ref.: 15/74/N. (665)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF CHEMISTRY

Applications for RESEARCH STUDENTSHIPS in all branches of Chemistry are invited from suitably qualified persons. The conditions and emoluments of the Studentships will broadly conform with those for S.R.C. awards.

Successful applicants will be required to register for a higher degree in the University of Aberdeen.

Applications should be addressed to Professor G. M. Burnett, Department of Chemistry, University of Aberdeen, Aberdeen AB9 2UE. (673)

LABORATORY TECHNICIAN GRADE 6, REquired in the Department of Physiology. The person appointed would be responsible under the Head of the Department and the Department Superintendent for the operation of teaching laboratory facilities and for arranging the availability of laboratories and equipment to coincide with the needs of teaching and practical classes in circulatory, electro, and respiratory physiology, allocation of, and subsequent supervision of, work schedules to technical staff, "on the job" training of junior technical staff as dictated by the varying needs of the department, assist with the general administration and maintenance of expenditure records. Applicant should hold H.N.C. or equivalent qualifications in relevant subjects and have had a minimum of nine years' experience in biological science work. Salary is in the range of £2,370 to £2,874 according to qualifications and experience.—Applications stating age, qualifications and full experience together with the names and addresses of two referees should be addressed to: Mr. E. French, Departmental Superintendent, Department of Physiology, Medical Multipurpose Building, Mount Preston Street, Leeds 2. (672)

Microbiologist Food Industry

We require a qualified microbiologist aged up to 25 to join the team at our research centre at Hayes Park, Middlesex.

He should preferably have had experience in the food or allied industries and his degree studies should have included a formal course in microbiology.

The work is concerned with the application of microbiological techniques to practical situations. Evolving methods and standards for ingredient materials, food in manufacture and finished packs. Setting sterilizations and pasteurisation processes.

Salaries and conditions are those of a large, progressive company.

Please write, giving brief details of background history to date, to:

The Personnel Officer (Ref. AD/91),
H. J. Heinz Company Limited,
Hayes Park, Hayes, Middlesex.
(644)

Heinz

IMPERIAL COLLEGE

Applications are invited for appointment of

POST-DOCTORAL RESEARCH ASSISTANT

to study radiation chemistry of osmotic membrane materials under supervision of Dr. P. G. Clay. Applicants should have good qualifications in physical and organic chemistry: Experience in radiation dosimetry is desirable. Initial salary in range £1,929-£2,388 per annum, plus £162 London Allowance and FSSU (in scale £1,929-£3,048).

Curriculum vitae and names and addresses of two referees to: Professor G. N. Walton, Department of Chemical Engineering and Chemical Technology, Imperial College, London SW7 2AZ. (585)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES

SCHOOL OF AGRICULTURE

Sutton Bonington, Loughborough, Leics.

Vacancies exist for postgraduates (including those expecting to graduate in 1974) to undertake research in the following fields.

"Reproductive physiology of poultry"
"Molecular mechanisms of growth control in plants"
"Phytochrome action in plants"
"Soil chemistry, physics and biology"
"Microbiology of plants and animals"

Further particulars can be obtained from the Administrative Assistant at the above address. (586)

UNIVERSITY OF DUNDEE

Department of Medical Biophysics

Applications are invited for a RESEARCH ASSISTANTSHIP tenable for two years. The work will involve an investigation into the effects of diagnostic and therapeutic ultrasound on the cell membranes of lymphocytes and thymocytes, and should provide suitable material for an M.Sc. thesis.

Candidates should possess a good Honours degree in Biochemistry or Biological Sciences. Starting salary will not be less than £1,200 per annum plus higher degree fees.

Applications, quoting Ref. Est/22/74J, and including the names of two referees, should be sent to the Secretary, The University, Dundee DD1 4HN, by March 1, 1974. (648)

AREA LABORATORY KING EDWARD VII HOSPITAL

WINDSOR

Applications for a BASIC GRADE/SENIOR GRADE BIOCHEMIST are invited. The position is post-doctoral or with equivalent clinical laboratory experience. The work entails development of and participation in new techniques related to automated reaction rate and C 300 Analysers. The appointee will be expected to study for the M.C.B.

The hospital is situated close to public termini, is within easy access of London by road and has its own swimming pool. Salary and conditions of service according to current Whitley Council agreements.

Apply to Consultant Biochemist, Area Laboratory, King Edward VII Hospital, Windsor, Berkshire. (681)

CHEMISTS

For Radiochemical Development and Production

The Radiochemical Centre Limited at Amersham supplies radioactive isotopes to doctors, scientists, technologists and other users worldwide.

For a number of different areas of work we seek graduate and postgraduate Biochemists and Organic Chemists to provide technological support for further expansion of output.

Alternatively you might undertake special preparations for customers or assist in the very challenging field of quality control development.

You should preferably be in your 20's and the scope of your academic background is more important than industrial experience since full training in the special work of the Centre will be given. Scientists with managerial aspirations will be encouraged to develop their skills.

Competitive salaries will be offered, plus fringe benefits – including the possibility of re-location assistance to a most attractive rural area of Buckinghamshire, yet within easy reach of London.

Write, giving full details of qualifications etc. to the Personnel Officer (interviews to suit academic or vacation commitments).

The Radiochemical Centre

Amersham Bucks (689)

BIOLOGIST

Male graduate (or HNC) biologist required to join a team working in respiratory physiology and inhalation toxicology. Studies involve primates and rodents in the areas of air pollutants and therapeutic aerosols. Salary within the range £1,400-£2,800, depending upon experience.

Application forms from Mrs. Nancy McCree, Huntingdon Research Centre, Huntingdon PE18 6ES. Telephone Woolley 431, Ext. 251.

(578)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BOTANY

Applications are invited for **two NERC Research Assistantships in Marine and Freshwater Algology.** The projects are on genetic variation and adaptation in a marine alga and on the influence of recovery from pollution on aquatic vegetation in a canal. Candidates should possess a good Honours degree in Botany or Biology, and some training in algology is desirable. Applications from final year undergraduates will also be considered.

Applications, together with the names of two referees, should be sent by February 28 to The Registrar, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, from whom further information may be obtained. Quote ref. RV/N/80685. (583)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

CANADA

ASSISTANT PROFESSOR IN BIOCHEMISTRY

Applications are invited for the post of Assistant Professor in the Department of Biochemistry (Head Designate, Dr. C. C. Bigelow), to take effect from September 1, 1974, or as soon thereafter as possible. The Department teaches undergraduates in the Faculty of Science and has an active postgraduate research and teaching programme. The successful applicant will be required to take part in the teaching duties of the Department and to develop his own research programme. The salary is negotiable, depending on experience.

Applications, including a curriculum vitae and the names of three referees, should be sent to Dr. E. A. Barnsley, Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, to reach him not later than March 31, 1974. (616)

UNIVERSITY OF TASMANIA

LECTURESHIP IN CLIMATOLOGY

Applications are invited for the above-mentioned post within the Department of Geography.

The salary scales are: Lecturer Grade II, $A8,698 to $A10,106; Grade I, $A10,575 to $A11,982. Appointment will be offered within one of these grades according to qualifications and experience.

Further details about the structure of courses, departmental staff, current research activities, together with conditions of appointment and application forms, are available from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close on March 15, 1974. (617)

MICROBIOLOGIST

UNIVERSITY OF GUELPH

Assistant Professor, initially contractually limited. Ph.D. with postdoctoral training and teaching experience. Duties include teaching and independent research in applied microbiology as it relates to microbial productivity.

Send curriculum vitae and names of three referees by June 15, 1974, to Dr. D. C. Jordan, Microbiology Department, University of Guelph, Guelph, Ontario, Canada N1G 2W1. (686)

THE INSTITUTE OF CLINICAL RESEARCH

MIDDLESEX HOSPITAL MEDICAL SCHOOL

RESEARCH TECHNICIAN

An additional research Technician is required to join a small team in expanding project to measure new peptide hormones. A knowledge of radioimmunoassay or similar techniques would be helpful. Salary according to experience or qualifications (maximum £1,800).

Please apply to Dr. S. R. Bloom, The Institute of Clinical Research, 40 Hanson Street, London, W.1. (695)

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF METALLURGY AND MATERIALS SCIENCE

University Demonstratorship

The Appointments Committee intend to appoint a University Demonstrator in the Department of Metallurgy and Materials Science. Applications are invited from graduates in Metallurgy, Materials Science or Physics, preferably with a Ph.D. and some teaching experience. It is proposed to fill the appointment in the field of newer non-metallic materials, with particular reference to their physical properties. Some experience outside a University would be useful, and candidates should be prepared to teach both basic and applied aspects of the subject.

The appointment is for a maximum tenure of five years on the scale £2,058 to £2,718 a year, with FSSU superannuation.

Applications (10 copies) should be sent to the Secretary, Appointments Committee of the Faculty of Physics and Chemistry, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE, not later than February 27, 1974. (579)

UNIVERSITY OF BATH

SOUTH-WEST UNIVERSITIES COMPUTER CENTRE

Applications are invited for the post of

DIRECTOR

of a newly established computer centre to be located at Bath.

The Centre will be equipped with a large new machine and will be linked to and provide a service for the Universities of Bath, Bristol and Exeter, University College Cardiff and the University of Wales Institute of Science and Technology, augmenting the existing network.

The person appointed will be on the staff of the University of Bath but responsible to the South West Universities for directing the work of the Centre staff, for giving technical advice and ensuring that the optimum use is made of the resources provided.

The salary would be in the professorial range at not less than £5,625 per annum.

Further particulars available from Registrar (S), The University, Bath BA2 7AY, quoting reference 74/7. (656)

Applications are invited for a Senior Scientific Officer at the Agricultural Research Council's Letcombe Laboratory, Wantage, Berkshire, to fill a vacancy for a

SOIL SCIENTIST

The successful candidate will be responsible for a Research Programme on gas movement in the soil in relation to soil physical conditions and plant growth. He will be expected to collaborate closely with existing experimental programmes on the effects of tillage and drainage.

Minimum qualifications: A Ph.D. in a relevant area of soil science and at least four years postgraduate experience of both field and laboratory work. Expertise in gas analysis and in field studies of the structure and moisture status of the soil is desirable.

Depending on experience the starting salary will be in the range £2,615-£3,640. Superannuation under FSSU with a non-pensionable allowance to offset personal contributions.

Applications to the Secretary, Letcombe Laboratory, Wantage OX12 9JT, Berkshire by March 1, 1974. (600)

SHEFFIELD NO. 3 HOSPITAL MANAGEMENT COMMITTEE

REGIONAL MEDICAL PHYSICS DEPARTMENT

PHYSICISTS

(Basic Grade) 2 Posts

(a) Required in the first instance in the instrumentation section concerned with equipment development and clinical applications in the use of electronics in medicine and physiological measurement.

(b) Required in the first instance in the section working in the field of radiation. Some experience in this field would be an advantage.

Good honours degree essential for both posts. In-post training will be given and there is a possibility of study for a higher degree.

Salary scale in the range £1,380 to £2,517 with starting point depending on qualifications and experience.

Applications naming two referees should be made to the Chief Physicist, Regional Medical Physics Department, Whitham Road, Sheffield S10 2SJ, from whom further details and an application form can be obtained. Closing date for applications February 22, 1974. (701)

NATIONAL RADIOLOGICAL PROTECTION BOARD

is seeking

SCIENTISTS

to fill vacancies in the Biology Department of the Research and Development Division.

The research programme involves studies of the metabolism of radioactive materials including the higher actinides. Their removal from the body tissues and their interaction with biological materials in cellular systems. The posts, which are at the Board's Headquarters at Harwell, would be of interest to Scientists trained in one of the biological sciences.

Knowledge of experience in histological techniques and cell kinetics would be useful. Previous experience of the chemistry of actinides is not essential.

Applicants should have a first, or good second-class, honours degree.

Appointments will be in the grade of Scientific Officer or Higher Scientific Officer, depending upon the level of qualifications and experience.

The salary scales appropriate to these grades are:

Scientific Officer	£1415–£2330 per annum
Higher Scientific Officer	£2225–£2855 per annum

For further information and application form please telephone or write to:

The Personnel Officer (A46/N),
National Radiological Protection Board,
Harwell, Didcot, Berkshire.
Telephone Rowstock 600 (STD Code 023-583) Extn. 33.

(589)

The Spastics Society

An exciting new project to further the education and to widen the life experiences of handicapped young people—

CHURCHTOWN FARM FIELD STUDY AND ADVENTURE HOLIDAY CENTRE,
LANLIVERY, CORNWALL.

WARDEN

required by late summer, 1974 to open this new project for handicapped young people and adults. The Centre, on a 5-acre site midway between Bodmin Moor and Fowey, will be purpose built with accommodation for 24 residents and additional staff.

The Warden must be well qualified in some branch of the natural sciences and be ready to develop a wide range of outdoor pursuits, including pony trekking, sailing, climbing, canoeing and camping.

It could be that a man and wife team will be appointed with one member acting as Housekeeper. A three-bedroom house is available on site.

Salary—within the range of £2,000–£3,000.

Further details and application forms from The Education Officer,
The Spastics Society, 12 Park Crescent, London, W1N 4EQ.

(687)

AGRICULTURAL RESEARCH COUNCIL, Unit of Statistics, University of Edinburgh, 21 Buccleuch Place, Edinburgh EH8 9LN.

SCIENTIFIC OFFICER required for a new post in this Unit which is attached to the Department of Statistics in the University of Edinburgh. The appointment will be for three years, and candidates should have a good honours degree in mathematics or statistics, with at least one year's postgraduate experience or similar qualification. Salary in scale £1,318 to £2,177 per annum according to qualifications and experience; superannuation under F.S.S.U.

Applications, with the names of three referees, should be sent by March 8, 1974, to the Director, from whom further particulars of the post may be obtained. (628)

The Council for Technical Education and Training for Overseas Countries is engaged in an increasing British aid programme in technical education and allied fields and intends to expand its staff by appointing an:

Assistant Adviser Agricultural Education and Training

£3,647 - £4,750

(under revision)

whose work for developing countries, conducted in close collaboration particularly with the Natural Resources Advisory Group of the Overseas Development Administration, will include:

- assessing needs, priorities and possibilities for education and training in agriculture and related fields
- planning, developing and monitoring associated training schemes and programmes
- advising on student and trainee placement in Britain
- maintaining close contact with relevant British education and training institutions and organisations.

Applicants for this London-based, pensionable post, which involves extensive overseas travel, should

- hold a good degree or equivalent qualification in agriculture or a related subject, with a recognised teaching diploma as an added advantage
- have experience in agriculture, teaching and administration at a responsible level, including significant overseas experience.

Apply by letter giving full details of education, qualifications and career to The Secretary, TETOC, 35/37 Grosvenor Gardens, London, SW1W OBS, quoting ref: AET/N

(629) **tetoc**

The Polytechnic of North London
Holloway, London, N7 8DB

NATIONAL COLLEGE OF RUBBER TECHNOLOGY

Immediate applications are invited for the following teaching appointments which provide opportunities for personal and team research in polymer science and technology:

Lecturer II in Plastics Technology

A qualified polymer chemist or chemical technologist with industrial experience of polymer processing technology.

Lecturer II in Polymer Engineering

A qualified engineer with industrial experience of polymer processing technology.

Salary scale: £2,515 × £91—£3,243 plus £118 London Allowance.

Apply for application form and further details, stating post in which interested, to The Deputy Establishment Officer, The Polytechnic of North London, Holloway Road, London N7 8DB.

(690)

DEPARTMENT OF CANCER STUDIES

TECHNICIANS (2)

Required for the Department of Cancer Studies to work on projects supported by the Cancer Research Campaign. Experience in Biochemistry, tissue culture or cytogenetics required.

Qualifications required to ONC/HNC or AIMLT level. Salary scale £1,650 to £1,920 per annum.

Applications, giving details of age, qualifications and experience, together with the names of two referees, should be sent to the Assistant Secretary (Personnel), University of Birmingham, P.O. Box 363, Birmingham B15 2TT, by March 11, 1974. REFERENCE 450/B/138.

(582)

UNIVERSITY OF KHARTOUM
SUDAN

Applications are invited for the following posts in the Faculty of Science:

1. SENIOR LECTURER/LECTURER IN APPLIED MATHEMATICS in Department of Mathematics.
2. SENIOR LECTURER/LECTURER IN SOLID STATE PHYSICS in Department of Physics.
3. SENIOR LECTURER/LECTURER IN OPTICS OR ELECTRONICS in Department of Physics.

Salary scales: Senior Lecturer £S2,400-£S2,800 p.a. Lecturer £S1,500-£S2,300 p.a. (£1 sterling=£S0.82). The British Government may supplement salaries in range £1,300-£1,600 p.a. (sterling) for married appointees or £800-£900 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages; various allowances; superannuation scheme; annual overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than March 6, 1974, to the Personnel Secretary, University of Khartoum, P.O. Box 321, Khartoum, Sudan. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (607)

UNIVERSITY OF KHARTOUM
SUDAN

Applications are invited for the post of SUPERVISOR OF THE ELECTRONICS INSTRUMENTATION WORKSHOP. Appointee would be on secondment for 2 years. Salary scale: £S2,400-£S2,800 p.a. (£1 sterling=£S0.82.) The British Government may supplement salary by £1,600 p.a. (sterling) for married appointee or £900 p.a. (sterling) for single appointee (normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages; various allowances; superannuation scheme; annual overseas leave.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than March 7, 1974, to the Personnel Secretary, University of Khartoum, P.O. Box 321, Khartoum, Sudan. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (610)

UNIVERSITY OF BOTSWANA, LESOTHO AND SWAZILAND

Applications are invited for a LECTURESHIP IN CHEMISTRY tenable at the Lesotho Campus to assist with the teaching of Physical Chemistry at the general B.Sc. degree level. Appointment may be permanent or on contract for limited periods, normally for two years in the first instance. Salary scale: R3,204-R4,764 p.a. (£1 sterling=R1.46.) A gratuity of 25% is paid in lieu of superannuation. The British Government may supplement salary in range £375-£600 p.a. (sterling) for married appointee or £150-£250 p.a. (sterling) for single appointee (normally free of all tax) and provide children's holiday visit passages. Education allowance; medical scheme; accommodation at reasonable rents; family passages; biennial overseas leave.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be forwarded by air mail, not later than March 12, 1974, to the Registrar, University of Botswana, Lesotho & Swaziland, Roma, Lesotho, Africa. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address.

CHAIRMAN—DEPARTMENT OF PHYSICS AND ASTRONOMY
UNIVERSITY OF OKLAHOMA

Applications are invited for the chairmanship of the Department of Physics and Astronomy, position available July 1, 1974. We are seeking and accomplished experimentalist with administrative interests. Rank and salary will depend on past experience. The successful candidate is expected to be active in one of the following areas: (1) Astrophysics, (2) Atomic and Molecular Physics, or (3) Low Temperature Physics. The Chairman's primary responsibility will be to (A) actively promote research by the faculty and (B) to ensure that the Department provides quality instruction. Interested persons are asked to send a resume and the names of three references before March 25 to: Chairman, Search Committee, Department of Physics and Astronomy, University of Oklahoma, Norman, Oklahoma 73069,.

Suggestions concerning suitable candidates are welcome.

O.U. is an equal opportunity and affirmative action employer. (598)

UNIVERSITY OF CAPE TOWN
CHAIR OF MINERALOGY AND GEOLOGY

Applications are invited for the Philipson-Stow Chair of Mineralogy and Geology, which will become vacant on February 1, 1974, through the appointment of Professor E. S. W. Simpson as Director of the National Institute of Oceanology. The closing date for applications is March 31, 1974.

CHAIR OF ZOOLOGY

Applications are invited for the Chair of Zoology, which becomes vacant on January 1, 1975, through the retirement of Professor J. H. Day. The Department of Zoology has a long tradition of research in Marine Biology, which the University is anxious to maintain. To this end, it is the intention of the University to appoint a Marine Biologist to the Chair. The closing date for applications is April 30, 1974.

The salary scale for both the above posts is R8,100 by R300 to R9,900 per annum, plus a 15 per cent pensionable allowance.

Applicants should submit a full curriculum vitae indicating their teaching and research experience and interests, present salary, and the earliest date on which they could assume duty. They should also give the names and addresses of three referees whom the University may consult.

Further information should be obtained from the Registrar, University of Cape Town, Private Bag, Rondebosch, C.P., to whom applications must be submitted by the closing dates stated above.

Appointment will be subject to a satisfactory medical certificate and the University reserves the right to appoint a person other than one of the applicants or to make no appointment.

(650)

DEPARTMENT OF BIOLOGY, COLLEGE OF SCIENCE
UNIVERSITY OF MOSUL
MOSUL, IRAQ

Applications are invited for teaching positions in chordates (vertebrate morphology), algae, archegonates and animal physiology for next academic year starting from September 1, 1974. Applicants should be holders of Doctorate degree and possess at least five years of university teaching and research experience in their respective fields. Salary will be fixed according to qualifications.

Detailed application, including a curriculum vitae and naming two referees, should be sent by airmail to Dr. M. H. Kasim, Head of the Department, at the earliest. (X426)

UNIVERSITY OF THE WEST INDIES
TRINIDAD

Applications are invited for posts of (a) LECTURER or (b) ASSISTANT LECTURER IN PHYSICS. Applicants should be interested in teaching and research in one or more of the following fields: Ceramics, Crystal Physics, Electronics. Salary scale: (a) TT$12,612 to TT$20,316 per annum; (b) TT$10,200 to TT$11,232 per annum. (£1 sterling= TT$4.8.) F.S.S.U. Family passages; triennial study leave.

Detailed applications (6 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail as soon as possible to the Secretary, University of the West Indies, St. Augustine, Trinidad. Further particulars for these posts will be sent to all applicants. (622)

UNIVERSITY OF CALGARY, GRADUATE Teaching and Research Assistantships are available **for students** proceeding to the **Ph.D.** or **M.Sc.** degrees in all branches of Chemistry and Biochemistry. The Assistantships are valued from $4,100 to $5,000 per annum and carry remission of programme fees and travel allowance.

For further information write to: Department of Chemistry, Calgary, Alberta T2N 1N4, Canada. Please indicate field of interest. (X35)

Glaxo

Graduate Microbiologist

A graduate in microbiology or a related discipline is required to join our chemotherapy team, a part of the Microbiology Department situated at Greenford, Middlesex. The team performs an important stage in the development of new antimicrobial agents, assessing the effectiveness of newly-synthesised compounds against a wide range of pathogenic micro-organisms.

This particular post will be chiefly concerned with the in vitro aspects of the work, and the successful applicant will have responsibilities which will include the development of new assay techniques. Trained assistants will be available to help in this work.

Preference will be given to those with relevant experience, although recent graduates will be considered.

Conditions of employment are attractive and include a London allowance, and pension and bonus schemes. In addition, the Department operates flexible working hours.

Please write for an application form to the Personnel Officer (Research GGP), Glaxo Laboratories Ltd., Greenford, Middlesex, quoting reference U.712 or ring 01-422 3434 ext. 200.

(654)

UNIVERSITY OF ZAMBIA

Applications are invited for posts of (a) CHIEF TECHNICIAN OR (b) SENIOR TECHNICIAN IN DEPARTMENTS OF OBSTETRICS AND GYNAECOLOGY and PATHOLOGY AND MICROBIOLOGY. Candidates must be in possession of at least AIMLT or equivalent. Exfoliative Cytopathology will be an added advantage for the post in the Department of Obstetrics and Gynaecology. Salary scales: (a) K3,300-K4,900 p.a. (b) K2,900-K3,500 p.a. (£1 sterling=K1.39.) Family passages; various allowances; superannuation and medical aid schemes; regular overseas leave.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be forwarded by air mail, not later than March 12, 1974, to the Registrar, University of Zambia, P.O. Box 2379, Lusaka, Zambia. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further particulars may be obtained from either address. (611)

UNIVERSITY OF ALBERTA
FACULTY OF SCIENCE
DEPARTMENT OF LINGUISTICS

Applications are invited for an ASSISTANT PROFESSORSHIP in Experimental Phonetics, commencing July 1, 1974. Salary range $13,125 to $17,198.

The department's terms of reference are to conduct research and teach in the field of experimental linguistics with some responsibility for university service teaching in general linguistics.

Preference will be given to a candidate with special research interests in the physiology of speech perception or speech production with ability to teach service courses in general linguistics. The Ph.D. is essential.

For further information, write to Professor C. I. J. M. Stuart, Head, Department of Linguistics, University of Alberta, Edmonton, Alberta, Canada T6G 2H1. (682)

UNIVERSITY OF SYDNEY
LECTURESHIP IN BIOPHYSICAL CHEMISTRY

Applications are invited for a fixed-term Lectureship in Physical Chemistry. The appointment will commence as soon as possible and will be for two years in the first instance. Preference will be given to candidates with special interests and research experience in biophysical chemistry.

Salary range: $A8,698 to $A11,982 per annum.

Applications, including curriculum vitae, list of publications and names of three referees, by March 15, 1974, to Registrar, University of Sydney, New South Wales 2006, from whom further information is available. Information also available from Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF. (623)

LIVERPOOL POLYTECHNIC, DEPARTMENT of Chemistry, Senior Lecturer/Lecturer II in Biochemistry. Required to teach general biochemistry to Honours Degree level and contribute to the Department's planning of new courses in this rapidly expanding area. A knowledge of modern biochemical techniques is essential. Teaching experience and expertise in the field of mammalian biochemistry would be an advantage. Applicants should have a first degree in Chemistry or Biochemistry and a higher research degree in Biochemistry. Lecturer II in Chemical Process Engineering. Applications are invited for the appointment of a chemical engineer to make a major contribution to the teaching of Industrial Chemistry at Honours Degree level and to exploit the potential of the custom-built industrial laboratory now being commissioned. Applicants should hold a first or higher degree in chemical engineering and industrial experience on plant would be an advantage. Salary: Senior Lecturer £3,291 to £3,928 per annum; Lecturer II £2,515 to £3,243 per annum.—Details available from Staff Office, Department (N), Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool L3 9RH. (651)

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

Plant Health and Seeds Inspectors

These posts in the Ministry's regional network are concerned with preventing the spread of dangerous pests and diseases and protecting the interests of consumers. The work, which involves direct contact with farmers, growers and seed merchants, includes inspection of plants and planting material entering or leaving the country, inspection of crops in the field and soil sampling. Initial specialised training will be followed by refresher courses.

Candidates (preferably aged 25–39) should normally have a degree (or equivalent) in Agriculture, Horticulture or related biological sciences *or* HND/National Diploma in either agriculture or horticulture. Others will be considered if they have a sound knowledge of agriculture or horticulture (preferably with some formal training) and hold GCE passes in English Language and 4 other subjects, including 2 at A-level of which one must be in a scientific subject. A current driving licence is essential.

Starting salary £1962, on scale (under review) rising to £2288. Non-contributory pension scheme and prospects of promotion.

For full details and an application form (to be returned by 4 March 1974), write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone BASINGSTOKE 29222 ext 500 or LONDON 01-839 1992 (24 hour answering service). Please quote S/8541.

(601)

THE QUEEN'S UNIVERSITY OF BELFAST

LECTURESHIP IN PALAEOECOLOGY

Applications are invited for a Lectureship in Palaeoecology in the Department of Botany from October 1, 1974. The salary scale is £1,929 to £4,548, with contributory pension rights under the F.S.S.U. Initial placing on the scale will depend on qualifications and experience. Applications should be received by March 5, 1974.

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland. (Please quote Ref. 74/N.) (640)

UNIVERSITY OF NEW BRUNSWICK

SAINT JOHN, NEW BRUNSWICK, CANADA

PHYSICIST. There is a vacancy for a Physicist at the Assistant Professor level within the Division of Science and Mathematics at the Saint John Campus of the University. The successful candidate will be expected to teach at the undergraduate level and to participate actively in the department's programme of research in low level gamma spectrometry and activation analysis. Candidates with qualifications in these areas will be preferred. Starting salary on the Assistant Professor scale is $11,500.

Applications (with curriculum vitae and names of two referees) should be sent as soon as possible to Dr. E. Stanley, Dean of Faculty, University of New Brunswick, Saint John, N.B., Canada E2L 4L5. (635)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF GENETICS

(DEVELOPMENTAL/ MOLECULAR BIOLOGIST)

A post-doctoral position is available immediately to work on messenger RNA processing in developing chick lens. The successful applicant will either be experienced in DNA-mRNA analysis, in DNA/RNA/protein molecular biology; subcellular organelle isolation, or high resolution analysis of proteins during development. Technical assistance will be available, and the appointee will be in charge of the day-to-day research programme, which will be carried out jointly in the Institute of Animal Genetics, University of Edinburgh, and the Beatson Institute for Cancer Research, Glasgow. The appointment will be for three years. Commencing salary £1,929 per annum. The starting date would be by agreement, but as soon as possible.

Interested applicants should send curriculum vitae and names of two referees to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL, not later than February 28, 1974. Please quote Reference Number 5002. (639)

LA TROBE UNIVERSITY

MELBOURNE, AUSTRALIA

LECTURER IN GEOLOGY

Applications are invited for the above-mentioned position. Special consideration will be given to those applicants with interests in geochemistry of sediments or statistical sedimentology.

Salary range: $A8,698 by $A470 (1) by $A469 (6) to $A11,982.

Further information and application forms are available from the Registrar, La Trobe University, Bundoora, Victoria, Australia 3083, with whom applications close on March 31, 1974. (620)

CHARING CROSS HOSPITAL MEDICAL SCHOOL

RESEARCH ASSISTANT required for up to 3 years in the Endocrine Section of the Department of Chemical Pathology to take part in an M.R.C. supported project to investigate certain endocrine aspects of breast cancer. The post is suitable for an A.I.M.L.T. or A.I.S.T. technician or recent graduate. Salary and conditions in accordance with Whitley Council regulations.

Applications, naming two referees, to Dr. J. R. Daly, Department of Chemical Pathology, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF.

(633)

UNIVERSITY OF MELBOURNE

LECTURER IN INVERTEBRATE BIOLOGY

IN THE DEPARTMENT OF ZOOLOGY

Qualifications: Ph.D. with some experience in teaching and in organising courses.

Duties: To contribute to the first year Biology course. To conduct or assist in Unit Courses in second and third years. The Lecturer will be encouraged to develop a personal research programme and to supervise graduate students.

The Department has excellent modern facilities for electron-microscopy (both transmission and scanning) electrophysiology, histo- and cyto-chemistry, tissue culture, bioacoustics and marine zoology. The Head of the Department is Professor G. Burnstock.

Salary range: $A8,698-$A77,982. The initial salary will be determined according to qualifications and experience.

Further information, including details of superannuation, travel and removal expenses, housing assistance and conditions of appointment, is available from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close on February 28, 1974. (663)

UNIVERSITY OF CAMBRIDGE

ANIMAL PRODUCTIVITY/NUTRITION

Applications invited for a vacant University Demonstratorship in Animal Productivity/Nutrition in the Department of Applied Biology. Qualifications—good Honours degree in animal sciences with research experience in some aspect of animal productivity involving nutrition and biometry and some teaching experience. Salary in scale £2,058 by £165 to £2,718. Starting point determined according to seniority. Pensionable under F.S.S.U. Limited contribution to removal expenses.

Further details from and applications including full personal particulars, list of publications and names and addresses of not more than three referees to the Secretary, Appointments Committee of the Faculty of Biology "A", Department of Applied Biology, Downing Street, Cambridge CB2 3DX, not later than February 28, 1974. (657)

AGRICULTURAL RESEARCH COUNCIL

POST-DOCTORAL RESEARCH CHEMIST

A post-doctoral Research Chemist is required for a project concerned with establishing mechanisms of degradation of organophosphorus insecticides in soil and vegetation. The work will be carried out at the Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire.

Applicants should have some experience in modern spectroscopic, chromatographic and other analytical techniques and have some background in organic chemistry.

The appointment, which will be for two years, will be as Higher Scientific Officer (£2,076 to £2,667 per annum) or Senior Scientific Officer (£2,615 to £3,640 per annum), the grade and starting salary depending on experience.

Application forms and further particulars are available from Mr. F. Morley, Agricultural Research Council, 160 Great Portland Street, London W1N 6DT, to whom completed forms should be returned by March 8, 1974. (669)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)

Denmark Hill, London SE5 6RX

SENIOR RESEARCH BIOCHEMIST required to join multidisciplinary group working in field of liver metabolism and drug hepatotoxicity. Ph.D. preferred. Facilities for higher degree. Salary within the range £2,385-£2,880 plus £126 London allowance.

Further particulars from Dr R. Williams, Liver Unit. Applications to the Secretary of the Medical School (N), not later than March 1, 1974. (661)

UNIVERSITY OF HULL

DEPARTMENT OF BIOCHEMISTRY

SENIOR EXPERIMENTAL OFFICER

Applications are invited for the post of Senior Experimental Officer in the Chemostat and Fermenter Unit of the Department which will become vacant on June 1, 1974. The person appointed will be responsible to the Head of Department for the operation of the Unit and will be expected to assist with research. Candidates should possess considerable experience of chemostat operation and ancillary services and possess a degree or equivalent qualification in biochemistry, microbiology or related fields. The S.E.O. salary scale is £2,223 by £69 to £2,292 by £126 to £3,174 by £99 to £3,273 with F.S.S.U. benefits.

Applications (five copies), together with the names of two referees, should be sent by March 9 to the Registrar, The University of Hull, Hull HU6 7RX, from whom full particulars may be obtained. (668)

M.R.C. CLINICAL RESEARCH CENTRE

(NORTHWICK PARK HOSPITAL)
WATFORD ROAD, HARROW, MIDDLESEX
HA1 3UJ

Division of Immunology require two post-doctoral or medical scientific workers.

Post A: to extend work on control mechanisms in immunology, taking as a starting point observations on immunological unresponsiveness in mice and the transient nature of the primary response.

Post B: to initiate basic studies on the aetiology of immunodeficiency disease in a setting in which clinical and laboratory aspects can be combined.

The appointment is for 3-5 years and the salary is dependent upon age and experience.

Further details and application forms may be obtained from Mrs. J. Tucker-Bull. Please quote ref. 118/1A/B and state position of interest. (542)

UNIVERSITY OF MANCHESTER

Applications invited from graduates in metallurgy, geology or physics to work on metallographic aspects of meteorite material using conventional metallographic methods in conjunction with a SEM 2 electron probe microanalyser. Appointment for one year in the first instance, renewable for up to three years. Salary range per annum: £1,929 to £2,388.

Applications (with curriculum vitae and names of two referees) to Dr. H. J. Axon, Department of Metallurgy, The University, Manchester M13 9PL. (699)

LONDON HOSPITAL MEDICAL COLLEGE

(DEPARTMENT OF PHYSIOLOGY)

QUEEN MARY COLLEGE

(DEPARTMENT OF CHEMISTRY)

UNIVERSITY OF LONDON

Applications are invited for a Scientific Assistantship tenable for one year, starting as soon as possible. The project, directed by Dr. R. Bonnett and Dr. J. Barrowman, involves an investigation of biliverdin isomer distribution to be carried out in laboratories at both Colleges. Candidates with a training in biochemistry, physiology or organic chemistry, and a general interest in medical science preferred.

Salary within scale £1,428 to £1,854 per annum, plus £162 London Allowance.

Applications in writing (giving qualifications, previous scientific experience, and names and addresses of two referees) to the Registrar (N), Queen Mary College, Mile End Road, London E1 4NS, as soon as possible. (615)

UNIVERSITY OF GLASGOW

DEPARTMENTS OF BOTANY AND CHEMISTRY

Applications are invited from post-doctoral bio-organic chemists for a research position under an M.R.C. project grant to Drs. C. J. W. Brooks (Chemistry) and W. A. Harland (Pathology) for biochemical studies of human atherosclerosis. The research depends on modern micro-analytical techniques, including combined gas chromatography-mass spectrometry. Experience in lipid or sterol chemistry would be advantageous. The post is available immediately. Initial salary in the range £1,929 to £2,223 per annum for suitably qualified candidates.

Applications with curriculum vitae, citing two referees, should be sent to Dr. C. J. W. Brooks, Department of Chemistry, University of Glasgow, Glasgow G12 8QQ. In reply please quote Ref. No. 3408 M. (684)

JUNIOR TECHNICIAN REQUIRED TO ASSIST with research on Amino Acid and Vitamin C metabolism. Salary according to age and qualifications.—Apply in writing to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. D.M.1. (674)

VICE PRESIDENT FOR HEALTH AFFAIRS

A large Eastern University announces the vacancy of the position of Vice President for Health Affairs, the principle university officer responsible for educational and service programmes in the Schools of Medicine, Dental Medicine, Veterinary Medicine, Nursing and allied medical professions and the two university-owned hospitals. Letters of inquiry, applications and nominations should be accompanied by a curriculum vitae.

Write Box 688, Nature, T. G. Scott & Son, Ltd., 1 Clement's Inn, London WC2A 2ED. (688)

UNIVERSITY OF DUBLIN Trinity College

JUNIOR LECTURER IN PHYSIOLOGY (HISTOLOGY)

Applications are invited for the above post. The successful applicant will be responsible for teaching human and mammalian Histology to medical students, and to a small number of science students. A research interest in histochemistry, neurocytology, or electron microscopy would be an advantage.

Salary scale: £1,969 by £126 to £2,473.

Marriage and children's allowances are paid, and there is a non-contributory F.S.S.U.-type Pension Scheme.

Further particulars and application forms may be obtained from:

The Staff Secretary
West Theatre,
Trinity College,
Dublin 2,

who will receive completed applications up to March 7, 1974. (649)

UNIVERSITY OF THE WEST INDIES

BARBADOS

Applications are invited for the post of SENIOR LABORATORY TECHNICIAN/LABORATORY TECHNICIAN in the Department of Chemistry. Applicants must have 5 subjects at Ordinary level, including English Language, Mathematics and Chemistry. For appointment to the senior post (graduate or equivalent) a degree or H.N.C. in a scientific subject is required. Appointee will be required to prepare and supervise laboratory classes. Salary scales: Senior Laboratory Technician (graduate or equivalent) EC$9,072 to EC$11,544 per annum; Senior Laboratory Technician (without degree or equivalent) EC$6,120 to EC$8,928 per annum; Laboratory Technician EC$4,080 to EC$6,108 per annum. (£1 sterling=EC$4.8.) Salary offered will be dependent upon qualifications and experience.

Applications should be made on the prescribed forms obtainable from the Secretary, University of the West Indies, Cave Hill Campus, Bridgetown, Barbados, to reach him as soon as possible. (626)

UNIVERSITY OF GLASGOW

A BIO-ORGANIC CHEMIST is required for a post-doctoral research position, supported by the S.R.C., for studies of sesquiterpenoid biosynthesis induced in capsicum fruits by fungi. The post is tenable for 2 years. Salary up to £2,058, depending on age and experience.

Applications should be sent to Dr. C. J. W. Brooks, Chemistry Department, University of Glasgow, Glasgow G12 8QQ. In reply please quote Ref. No. 3406M. (645)

UNIVERSITY OF MANCHESTER

RESEARCH ASSISTANT IN OCCUPATIONAL HEALTH

Applications invited for this post, for experimental work on the effects of electric currents on the heart. Applicants should have a degree (or equivalent) in medicine, physiology or biology, and an interest in experimental work. Opportunity to work for a higher degree. Salary up to approximately £1,000 per annum, dependent upon age, experience and qualifications. Particulars and application forms (returnable by February 28) from the Registrar, The University, Manchester M13 9PL. Quote ref.: 19/74/N. (631)

UNIVERSITY OF BRISTOL

LONG ASHTON RESEARCH STATION

SCIENTIFIC OFFICER

required in the Biometrics Section to help in applying multivariate and related techniques to various Station experiments, particularly those on perennial crops. Also to assist research workers with data analysis problems.

Minimum qualifications pass degree, H.N.C. or equivalent in Statistics or Mathematics, or in a Biological subject with some mathematical ability.

Appointment in the Scientific Officer grade (£1,318 to £2,177 per annum), commencing salary according to qualifications and experience. Optional superannuation scheme with non-pensionable allowance.

Applications to the Secretary, Long Ashton Research Station, Long Ashton, Bristol BS18 9AF, by March 11, 1974. (696)

KINGSTON POLYTECHNIC

SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES

RESEARCH FELLOW IN ENVIRONMENTAL STUDIES

Applications are invited from suitable candidates to work with a group of staff on a multi-disciplinary study of pollution in selected estuaries. The work will be co-ordinated by Dr W. O. George, Reader in Spectroscopy.

Candidates with a degree in the Natural, Physical or Earth Sciences will be considered.

Salary on scale £1,778-£2,803 according to age, qualifications and experience.

Further details and application forms from Appointments Officer, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames, KT1 2EE (01-549 1366). (596)

UNIVERSITY OF KHARTOUM

SUDAN

Applications are invited for (a) SENIOR LECTURESHIP or (b) LECTURESHIP IN PHYSICAL GEOGRAPHY. Salary scales: (a) £S2,400-£S2,800 p.a. (b) £S1,500-£S2,300 p.a. (£1 sterling=£S0.82.) The British Government may supplement salaries in range £1,300-£1,600 p.a. (sterling) for married appointees or £800-£900 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages; various allowances; superannuation scheme; annual overseas leave.

Detailed application (6 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than March 6, 1974, to the Personnel Secretary, University of Khartoum, P.O. Box 321, Khartoum, Sudan. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London WIP 0DT. Further particulars may be obtained from either address. (612)

UNIVERSITY OF CAMBRIDGE

RESEARCH IN DATA-HANDLING FOR MUSEUMS AND GEOLOGY

TWO RESEARCH ASSISTANTS are required at the Sedgwick Museum, Cambridge, for a research project into computer-based data management systems for museums and geological data. One is required to assist in the development, testing and promotion of interdisciplinary standards for museum data, and to represent the practical side of museum activities during specification of a new programme package. Applicants should be experienced in museum work or qualified information workers interested in gaining experience of museum data-handling. The other should be a geologist with computing experience, to assist in building computer files for the Sedgwick Museum collections, and in the design and creation of a data base management system for a research project in sedimentology.

Salary in range £1,752 to £3,378 (F.S.S.U.). Apply to Dr. J. L. Cutbill, Department of Geology, Downing Street, Cambridge. (703)

RESEARCH TECHNICIAN REQUIRED TO assist in our investigation of the effects of radiation on the vascular system of the central nervous system. Previous laboratory experience desirable but not essential.—Further details from Dr. J. W. Hopewell, Research Institute, Churchill Hospital, Oxford; applications to the Secretary of the Board of Management, Research Institute. (678)

APPLICATIONS ARE INVITED FOR A Graduate Research Assistant (B.Sc.) at the Charles Salt Research Centre, The Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, Shropshire, to take part in a research programme concerning biochemical and biological aspects of Musculo-skeletal conditions. Commencing salary for candidates with 1st or 2nd Class Honours Degrees £1,680, other applicable Science Degrees £1,380.—Applications with details of degrees and experience to be sent to Mr. N. W. Nisbet, F.R.C.S., Director of Research, Charles Salt Research Centre, The Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, Shropshire SY10 7AG. (679)

British Museum (Natural History)

Zoologist

■ **In Crustacea Section** ■ **Undertake research into larval development of Decapoda** ■ **Curatorial and advisory work.**
☐ 1st/2nd hons degree in Zoology ☐ At least 2 years' postgraduate experience ☐ Experience in Crustacean or other larval invertebrate work ☐ Knowledge of laboratory culture techniques an advantage ☐ Age under 32 ☐ Appointment as Senior Scientific Officer (£2790–£3815) or Higher Scientific Officer (£2251–£2842) according to age and experience ☐ Ref: SB/16/DK.
☐ Application forms (for return by 8 March 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Science group
CIVIL SERVICE

(693)

A.R.C. ANIMAL BREEDING RESEARCH ORGANISATION

ANIMAL GENETICIST

Applications are invited for an ANIMAL GENETICIST with experience in quantitative genetics and the formulation of animal breeding plans. A good knowledge of biometrics and experience in computing are essential. Animal production experience under British conditions desirable.

The scientists appointed will head a small section devoted to the study of theoretical and applied breeding problems in a variety of areas. The work will include the planning and analysis of various animal breeding experiments to be carried out on experimental farms and in the field; examination of the economic implications of breeding plans and theoretical studies on improvement methods and analytical techniques.

An appointment in the P.S.O. grade is envisaged for suitably qualified and experienced applicants.

Salary: £3,472 to £4,575, with membership of F.S.S.U. and 4¼ per cent compensatory allowance.

Application, with names of three referees, should be sent to the Secretary, A.R.C. Animal Breeding Research Organisation, West Mains Road, Edinburgh EH9 3JQ.

(592)

Histopathologist

The **HUNTINGDON RESEARCH CENTRE** is Europe's largest contract research unit operating in the biological sciences area. We require a PATHOLOGIST to work in our Department of Pathology with a team of experienced pathologists—he or she would report directly to the Head of Department. The Department of Pathology provides a service to our research groups working in the fields of toxicology, teratology, pharmacology and environmental physiology. Much of the work is carried out in close co-operation with these departments and opportunities for general publication are encouraged. Applicants should have at least one year's experience in histopathology and knowledge or experience of comparative pathology would be advantageous. They will possess a medical or veterinary qualification though other suitably qualified candidates would be considered. The Centre provides opportunities for progressive development of expertise and contribution and can offer salary and benefits consistent with its status in a major International group. We are situated in a pleasant but convenient rural environment just outside Huntingdon within easy reach of Cambridge.

Further information on this position can be obtained from Dr W. Proudlock, Director of Personnel, Huntingdon Research Centre, Huntingdon, PE18 6ES, telephone Woolley 431, Ext. 230.

(577)

UNIVERSITY OF BRITISH COLUMBIA

DEPARTMENT OF CHEMISTRY

FACULTY POSITIONS

Applications are invited for positions at the rank of Assistant Professor. Applicants should be well-qualified, and those with postdoctoral experience in the fields of experimental Inorganic, Physical or Bio-Physical will be given preference. Date of appointment is open.

Interested applicants should send a full curriculum vitae and the names of three referees to: Professor C. A. McDowell, Department of Chemistry, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5.

(556)

CENTRAL PUBLIC HEALTH LABORATORY

We have a part-time vacancy in our Salmonella and Shigella Reference Laboratory for an HONOURS SCIENCE GRADUATE, preferably in microbiology, to work in the E. Coli-Shigella section. Hours by arrangement.

Applications to the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT.

(680)

UNIVERSITY OF THE WEST INDIES

TRINIDAD

Applications are invited for the post of SEISMOLOGIST (Research Fellow/Junior Research Fellow) in the Seismic Research Unit. Applicants should be graduates in Geophysics, Physics or Geology and have research experience in Geophysics. Salary scales: Research Fellow TT$12,612 to TT$20,316 per annum; Junior Research Fellow TT$10,200 to TT$11,232 per annum. (£1 sterling=TT$4.8.) F.S.S.U. Family passages; triennial study leave.

Detailed applications (6 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail as soon as possible to the Secretary, University of the West Indies, St. Augustine, Trinidad. Further particulars will be sent to all applicants.

(624)

IMPERIAL COLLEGE

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for the post of RESEARCH ASSISTANT in an inter-departmental research service laboratory. The work involves determinations of the structure and properties of surfaces (mainly non-biological materials) using modern instruments for electron microscopy and ESCA. The appointment will be for one year in the first instance, and is renewable. Initial salary in the range £1,929 to £2,388 per annum plus £162 London allowance and F.S.S.U. (in scale £1,929 to £3,048).

Applications with curriculum vitae, details of experience in this field, and the names and addresses of two referees, should be sent to: Professor A. R. Ubbelohde, C.B.E., F.R.S., The Department of Chemical Engineering and Chemical Technology, Imperial College, London SW7 2AZ.

(630)

UNIVERSITIES OF LONDON AND GLASGOW

UNIVERSITY MARINE BIOLOGICAL STATION, MILLPORT

Applications are invited for the post of LECTURER or SENIOR LECTURER. Applicants must have a general interest in marine ecology. Applications will be welcomed from those with a particular interest in the physiology or behaviour of marine organisms, fisheries, meiobenthos, marine and estuarine pollution, or sedimentology, but especially from those with an interest in marine microbiology.

Salaries: Lecturer, in the range £1,929 to £4,548; Senior Lecturer, £4,368 to £5,496 per annum, according to qualifications and experience.

Applications (original and 3 copies) including the names and addresses of 3 referees should be received not later than March 22 by the Secretary, University Marine Biological Station Millport, KA28 0EG, Cumbrae, Scotland, from whom further particulars may be obtained.

(638)

EAST MALLING RESEARCH STATION

PLANT VIROLOGIST to support a research team investigating virus diseases of fruit plants. Applicants must have a degree or H.N.C. in a biological subject. Experience in virus research an advantage. Salary on the Scientific Officer (£1,318-£2,177) or Higher Scientific Officer (£2,076-£2,667) scales, with non-pensionable allowance to offset superannuation contributions.

Details and application forms from the Assistant to the Secretary, East Malling Research Station, Maidstone, Kent ME19 6BJ.

(627)

THE LONDON HOSPITAL MEDICAL COLLEGE

(UNIVERSITY OF LONDON)

TECHNICIAN—IMMUNOLOGY

Applications are invited for the post of Technician in the Department of Immunology. Candidates should have obtained H.N.C. or equivalent but applications from graduates are also welcome. Experience/interest in tissue and animal work essential. Initial salary according to qualification and experience within the range £1,440/£1,845 plus £126 London Weighting per annum, together with superannuation.

Applications to the Secretary, The London Hospital Medical College, Turner Street, London E1 2AD, quoting IM/2/74, within 14 days.

(697)

LIVERPOOL POLYTECHNIC

School of Pharmacy Research

ASSISTANT IN ENZYMOLOGY

Applications are invited for the above post with a group investigating the structure and physical/chemical properties of clinically important enzymes and enzyme derivatives. Candidates should have or expect to obtain a good first degree or equivalent in chemistry, biochemistry, pharmacy or a related area. The successful appointee will be expected to work for a higher degree. Salary: £1,353-£1,644 p.a.

Details available from: Staff Office, Dept 'N', Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool L3 9RH.

(590)

FELLOWSHIPS AND STUDENTSHIPS

UNIVERSITY OF DUNDEE

RESEARCH FELLOWSHIP IN NUMERICAL ANALYSIS

Applications are invited from candidates with Ph.D. or equivalent qualifications for a S.R.C. Fellowship in Numerical Analysis, to be held in the Mathematics Department, which already has established interests in numerical analysis and is now developing an active group in optimisation. The appointment is for 3 years from October 1, 1974, or earlier, and carries a salary at the lower end of the University Lecturers' scale.

Applications, quoting Ref. Est/21/74J, and including the names of two referees, should be sent to the Secretary, The University, Dundee DD1 4HN, from whom further particulars may be obtained. (647)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF CANCER STUDIES

POSTGRADUATE STUDENTSHIPS

Applications are invited for two research studentships leading to the degree of Ph.D., tenable for three years. The subject of the research would be some aspect of the relationship between tumour viruses and the host cell genome. The studentships are supported by the Cancer Research Campaign. Good facilities exist for virology, tissue culture, cytogenetics and molecular hybridization. The value of the grant will be £695 per annum, and the conditions of award similar to those for S.R.C. and M.R.C. awards.

Applications with curriculum vitae and names of two referees to Professor D. G. Harnden, Department of Cancer Studies, Medical School, Birmingham B15 2TJ, by March 11, 1974. (531)

UNIVERSITY OF EXETER

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a postdoctoral fellowship for work on the biosynthesis of Bisbenzylisoquinoline Alkaloids, and on related Phenol Oxidations.

Appointment will be for two years commencing as soon as possible. Salary on the scale £1,929-£2,058-£2,223 per annum with placements according to experience. The post is superannuable (FSSU).

Applications, with the names of two referees, should be sent to Professor K. Schofield and Dr D. H. G. Crout, Department of Chemistry, The University, Exeter EX4 4QD, by March 15, 1974. (588)

POSTDOCTORAL FELLOWSHIPS

Post-doctoral Fellowships at McMaster University are available in Biochemistry. The areas covered are: Mechanism of action of pituitary hormones, subunit structure and mechanism of regulatory enzymes, adaptations in the metabolism of peptide and protein hormones in pregnancy and lactation, biogenesis of mitochondria and mitochondrial RNA, mechanism of protein synthesis, host-virus interaction in cultured animal cell systems, tRNA structure and function, molecular events underlying growth and development of organisms, the regulation and behaviour of folate enzymes in cultured mammalian cells, mechanism of action of mutagens and carcinogens, synthesis of oligoribonucleotides of biological significance.

The fellowships are valued at $8,900 per year, taxable and may be renewed for one year. For further information write to: Dr. K. B. Freeman, Chairman, Department of Biochemistry, McMaster University, 1400 Main Street West, Hamilton, Ontario L8S 4J9, Canada. (637)

INSTITUTE OF PSYCHIATRY

RESEARCH STUDENTSHIPS IN NEUROCHEMISTRY

Applications are invited for MRC Training Scholarships leading to a Ph.D. in Neurochemistry. An upper second or first class degree is required. Research topics include studies on metabolic or cyclic AMP, neurotransmitters and energy intermediates in cerebral cortex preparations, subcellular fractions and nerve cells in tissue culture.

Applications should be made to the Secretary, Department of Biochemistry, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, U.K. (603)

Science Research Council

ROYAL OBSERVATORY EDINBURGH

SENIOR RESEARCH FELLOW

in the Astrophysics Division

Applications are invited for a Senior Research Fellowship in the astrophysics division at the Royal Observatory, Edinburgh. It is likely that the successful applicant will be associated particularly with the Observatory's infrared group which is currently developing photometric and interferometric equipment for work in both the near and far infrared, but that he will be primarily interested in some observational/theoretical aspect of galactic/extragalactic astronomy. Good opportunities exist for observational work at overseas sites particularly on the 60-inch flux collector at Tenerife, and it is expected that the successful candidate will take advantage of these opportunities.

Candidates should have a good honours degree in astronomy, physics or mathematics and research experience in astrophysics.

The fellowship will be for three years and will receive a fixed salary determined according to experience and merit between £2,611 and £3,252 per annum. There is a possibility of appointment to a permanent post at a later date. Provision will be made for F.S.S.U.

Application forms from

THE SECRETARY,
ROYAL OBSERVATORY
BLACKFORD HILL,
EDINBURGH EH9 3HJ
Telephone number 031-667-3321

Closing date for receipt of applications 1st March, 1974.

(606)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF PRODUCTION ENGINEERING

RESEARCH FELLOWSHIP IN ELECTROCHEMICAL MACHINING

Applications are invited for a Research Fellowship, supported by the S.R.C., in electrochemical machining under the guidance of Dr C. N. Larsson. The interdisciplinary investigation is to establish the reliability of the process and includes consideration of electrolyte cavitation, flow patterns, mass transport limitations and machine structure.

The Fellowship will be offered to an applicant with industrial or research experience and is for a period of three years with an initial salary up to £2,079 with F.S.S.U. benefits.

Applications (quoting R4/74) giving full academic qualifications, details of experience, and the names of two referees, should be sent to Professor D. S. Ross, Department of Production Engineering, University of Strathclyde, James Weir Building, 75 Montrose Street, Glasgow G1 1XJ. (595)

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THE WELLCOME TRUST

TRAVELLING RESEARCH FELLOWSHIPS

TO

WESTERN AND EASTERN EUROPE

1974/75

Applications are invited from British research workers for the award of Wellcome-European Research Fellowships for 1974/75. These awards include the Trustees' special fellowships to Denmark, Germany, Hungary, Norway and Sweden.

The object of these fellowships is to encourage working visits to countries in Western and Eastern Europe by investigators in any branches of the natural and clinical sciences which have a bearing upon human or animal medicine. Candidates whose research is related to clinical problems will be given preference. These fellowships, which are normally for one year, are intended for graduates from the United Kingdom who already have some **post-doctoral** research experience. Stipends, according to age and experience, are within the range of £1,930 to to £3,875 per annum. A special cost of living allowance applicable to the country in which the candidate wishes to work is given where necessary. Travelling and some incidental expenses are provided in addition.

The candidate must make his own arrangements with the department in which he proposes to work, and written evidence must be submitted confirming his arrangements. Application forms may be obtained from the **Assistant Director, The Wellcome Trust, 52 Queen Anne Street, London W1M 9LA.** Completed application forms must be returned by March 31, 1974.

(581)

AUSTRALIA

Queen Elizabeth II

FELLOWSHIPS

in the Physical and Biological Sciences

To commemorate the Royal Visit to Australia in 1963 the Australian Government established the Queen Elizabeth II Fellowships Scheme. Under this scheme up to ten fellowships may be awarded each year for full-time research by young scientists of exceptional promise and proved capacity for original work. These are post-doctoral awards tenable in an Australian university or approved research institution, normally for two years. Tenure of a Fellowship will commence on a date which normally should be within nine months of the date of the award.

QUALIFICATIONS Queen Elizabeth II Fellows must be either Australian or United Kingdom citizens. They should have a Ph.D., or equivalent qualifications, in one of the physical or biological sciences (which are deemed to include mathematics and the scientific aspects of statistics, engineering, metallurgy, agriculture and medicine).

Awards will, in general, be restricted to applicants who are not more than 30 years of age on the date when applications close.

STIPEND $9,500 (Australian) per annum – increased to $10,250 per annum at age 28 years.

ALLOWANCES are payable in respect of a Fellow's wife ($500 p.a.), each dependent child ($200), superannuation payments (up to 10 per cent of stipend), appropriate insurance coverage and necessary travel expenses. Host institutions are paid an allowance towards the cost of setting up a Fellow and his research work.

APPLICATIONS Persons interested in applying for the above fellowships should obtain application forms and a statement of the conditions of award from the Secretary, Queen Elizabeth Fellowships Committee, Department of Science, P.O. Box 449, Woden, A.C.T. 2606, Australia; The Education Liaison Officer, Canberra House, Strand, London, W.C.2, England; or The Consul General, Australian Consulate General, 636 Fifth Avenue, New York 20, N.Y., 10020, U.S.A. Applications for the next round of awards, which will be announced in JUNE, 1974, close at the Canberra address on 1 MARCH 1974.

(700)

THE OPEN UNIVERSITY

RESEARCH STUDENTSHIP IN BIOLOGY

Applications are invited for a **Research Studentship in Biology**, tenable from April 1, 1974, or as soon as possible thereafter, to work with Dr. Gary Dutton of the Brain Research Group on glycoprotein metabolism in the brain. Applicants should possess a 1st or good 2nd class honours degree in biochemistry or have equivalent neurochemical experience. The basic value of the Studentship will be £695 per annum. Appropriate additional allowances will be paid. The successful candidate will be expected to register for a higher degree of the Open University.

Further details are available from the Higher Degrees Office, The Open University (RB2), P.O. Box 49, Milton Keynes MK7 6AD. Closing date for applications: Friday, March 1, 1974. (625)

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHYSICS

A RESEARCH STUDENTSHIP

sponsored by the National Physical Laboratory is available for the study of a new method of precision thermometry between 2 and 20 K based on the dielectric properties of helium gas. The Studentship is available for three years from October 1, 1974, at a rate which will be rather higher than an S.R.C. Research Studentship. The starting date could be advanced for a qualified applicant.

The work will be suitable for submission for the degree of Ph.D. Applicants should preferably hold a first or upper second class Honours degree from a United Kingdom university, and must have an interest in and an aptitude for the use of precision measuring apparatus.

Applications should be sent to Dr. D. Gugan, Physics Department, Tyndall Avenue, Bristol BS8 1TL. (655)

UNIVERSITY OF NEW SOUTH WALES

SCHOOL OF PHYSIOLOGY AND PHARMACOLOGY

POST-DOCTORAL RESEARCH FELLOW

To investigate effects of funnel web spider and sea snake venoms on the secretion and postsynaptic effects of neuro-transmitters. Previous experience with microelectrodes desirable. Salary between $A7,556 and $A8,698 per annum according to qualifications and experience. Appointment is for one year from August, 1974.

Details of appointment may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close in Australia and London on March 16, 1974. (618)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES

ANIMAL PHYSIOLOGY SECTION

Applications are invited for Ph.D. STUDENTSHIPS for work on the control of metabolism in the lactating mammary gland. Projects currently in progress are concerned with amino acid and fatty acid utilization by isolated perfused guinea-pig glands.

Candidates should have, or expect to obtain this year, a 1st or upper 2nd class Honours degree in Biological Science, preferably with experience in biochemical and/or physiological techniques.

Enquiries should be addressed as soon as possible to Dr. T. B. Mepham, University of Nottingham, Faculty of Agricultural Science, Sutton Bonington, Loughborough, Leicestershire LE12 5RD.

(698)

GRANTS & SCHOLARSHIPS

IMPERIAL CANCER RESEARCH FUND

Bursaries for Training in Research

The Council of the Fund will award a limited number of Bursaries at the Laboratories in Lincoln's Inn Fields and Mill Hill for full-time studies leading to Higher Degrees in research fields relevant to cancer, including Cellular Biology, Endocrinology, Virology and Macromolecular Chemistry.

The award will be tenable for three years from October 1, 1974, with a non-superannuable grant of £1,270 a year (subject to tax), with, in some cases, additional allowances.

Applications are invited from British subjects, not over 25 years of age, normally resident in the UK, who hope to have First or Upper Second class degrees in Science in 1974.

Further details and application forms are obtainable from: **The Personnel Officer, Imperial Cancer Research Fund Laboratories, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX.** Applications to reach the Personnel Officer at the above address not later than March 22, 1974.

(683)

UNIVERSITY OF LEICESTER

RESEARCH SCHOLARSHIPS

Applications are invited for a number of Research Scholarships in ARTS, SCIENCE (including Engineering), LAW, MEDICINE, and the SOCIAL SCIENCES, tenable at the University for one year initially at a maximum annual value of £695 plus fees. Research Scholars may have the opportunity of acquiring some part-time University teaching experience.

Particulars and application forms may be obtained from the Registrar, to whom completed applications must be submitted by May 1, 1974. (518)

THE CAMILLA SAMUEL FUND

For the academic year 1974/75, the Trustees of the above Fund invite applications from research workers in medicine and allied disciplines. Consideration will be given to any research dealing with the causes, prevention and treatment of illness, and this year special consideration will be given to applications relating to research into arthritis, rheumatism, and allied disorders. The award, which will be made annually, will not normally be for more than three years. Application forms are available from the Secretary of the Trustees, W. G. Bayley, Esq., 100 Wood Street, London EC2P 2AJ, to whom they should be returned not later than May 31, 1974. (634)

UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY

RESEARCH IN CHEMISTRY

Research grants are available for British students, at postgraduate, postgraduate R.I.C. level for research in the following areas.

(1) Medicinal Chemistry.
(2) Heterocyclic and Aromatic Chemistry.
(3) Organic Reaction Mechanisms.
(4) Free Radical Chemistry.
(5) Carbene Chemistry.
(6) Organic Photochemical Reactions.
(7) Synthetic Organic Chemistry.
(8) Organo-sulphur Chemistry.
(9) Organometallic Chemistry.
(10) Metal Carbonyls.
(11) Organosilicon Chemistry.
(12) Organophosphorus Chemistry.
(13) Petrochemical Research.
(14) Polymer Synthesis.
(15) Valence-bond Isomers.
(16) Application of the Woodward-Hoffmann Rules.
(17) Chemical Process Development.
(18) Inorganic Polymers.
(19) Synthesis of perfumery and flavouring chemicals via cycloaddition reactions.
(20) Medicinal Applications of transition metal complexes.
(21) Energy transfer in porphyrins and metalloporphyrins.
(22) The stabilisation of novel metal-olefin bonds and their reactivity.
(23) Mechanistic, kinetic and structural studies on Ziegler-Natta and related stereospecific polymerization processes.
(24) New classes of transition metal catalysts based on polymeric substrates.
(25) Combustion and Flame.
(26) Heterogenous Catalysis.

Postgraduate, etc. grants range from £850 to £1,100 per annum depending upon the topic.

M.Sc. COURSE IN PETROCHEMICALS AND HYDROCARBON CHEMISTRY

The next course, which leads to the M.Sc. Degree of the University of Manchester, starts on October 1, 1974, and is open to Graduates in Chemistry or Chemical Engineering and to those with Grad.R.I.C., Dip.Tech. or equivalent qualifications at an appropriate level of achievement. It consists of lectures, seminars, projects, tutorials, practical work, and a short research project, and some of the lectures are given by distinguished speakers from industry. The course, which is designed for those interested in petrochemistry, in the chemistry of petroleum, or in general hydrocarbon chemistry, has received support from the Petroleum and Chemical Industries, and is approved by the Science Research Council.

Applications should be sent as soon as possible after the appearance of this advertisement to: Professor R. N. Haszeldine, F.R.S., Department of Chemistry, U.M.I.S.T., Manchester M60 1QD. (643)

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COURSE IN MACROMOLECULAR TECHNIQUES OF USE IN MEDICAL RESEARCH

of interest to medically qualified personnel will be held from July 1 to 4, 1974. Course fee £10. Accommodation available. Approved by Regional Postgraduate Education Committee for study leave pay and expenses.

Further details: Dr. R. L. Turner, M.D., F.R.C.Path., Chairman, Postgraduate School of Studies in Medical and Surgical Sciences, University of Bradford, Bradford BD7 1DP. (Closing date: April 11, 1974.) (636)

UNIVERSITY OF GLASGOW

ZOOLOGY DEPARTMENT

M.SC. IN ENTOMOLOGY

The course runs for two years (this may be reduced to one year for well qualified candidates) and starts on October 1, 1974. The syllabus covers both pure and applied Entomology. The teaching is by lectures and seminars and guided reading. The student undertakes a research project, the results of which are presented in the form of a thesis for the final examination after the conclusion of the course.

Intending applicants should write in the first place to the Secretary, Zoology Department, Glasgow University, G12 8QQ, from whom application forms can be obtained. The closing date for applications is May 1, 1974. (646)

FRESHWATER BIOLOGICAL ASSOCIATION

River Laboratory

A short Specialist Course on

THE SAMPLING OF AQUATIC MACROPHYTES OF FLOWING WATERS

A one-week intensive course will be held from May 30-June 5, 1974, on the theory and practice of sampling plants.

Full details from: The Secretary, Freshwater Biological Association River Laboratory, East Stoke, Wareham, Dorset BH20 6BB (Telephone: Bindon Abbey 462 314). (580)

INSTITUTES OF PSYCHIATRY, NEUROLOGY AND OPHTHALMOLOGY

(British Postgraduate Medical Federation, University of London)

POSTGRADUATE COURSE IN NEUROCHEMISTRY

This is a ONE-YEAR course leading to the degree of M.Sc. in Neurochemistry. It offers graduates in science and medicine training in basic and applied neurochemistry. The course consists of lectures, seminars and laboratory work and, in the second and third terms, research on a neurochemical topic.

Applicants should have an honours degree in Biochemistry or a Biological subject with some Biochemical component, or be medically qualified. The course is recognised by the Medical Research Council as an Advanced Course for the award of Training Scholarships to suitably qualified applicants.

Application for the Academic year October 1974-July 1975 should be addressed to the Secretary, Course in Neurochemistry, Department of Biochemistry, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, England. (602)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF MEDICAL PHYSICS

M.Sc. IN MEDICAL PHYSICS

The Department provides a 12 months' course in Medical Physics, commencing October 1 each year. Candidates should have a first or second-class honours degree in Physics or Electronics or Engineering, or equivalent qualifications.

The course covers a wide range of the applications of physics to medicine and is designed to prepare candidates for a career in Medical Physics.

The Science Research Council and Medical Research Council have accepted the course as suitable for the tenure of their Advanced Course Studentships. The course has been endorsed by the Departments of Health in London and Edinburgh.

Further details available from the Secretary, Department of Medical Physics, University of Aberdeen. (X77)

UNIVERSITY OF LEICESTER

M.Sc. COURSE IN EXPERIMENTAL SPACE PHYSICS

Graduates, and prospective graduates, in Physics or Engineering are invited to apply for admission in October 1974 to this one-year course in Experimental Space Physics leading to the M.Sc. degree.

The course of lectures and laboratory work will provide a survey of current researches in Space Physics and will include a comprehensive treatment of the techniques employed in space research. Some industrial studentships will be available and the course is also recognised by the Science Research Council as suitable for the award of its advanced course studentships.

Full particulars and application forms may be obtained from the Registrar, quoting PG5. (517)

THEORY AND APPLICATIONS OF ELECTROMAGNETIC SCATTERING

A three-day course of post-experience lectures will be held at IMPERIAL COLLEGE from Tuesday, April 2, to Thursday, April 4, 1974.

Further information from Dr. A. R. Jones, Department of Chemical Engineering and Chemical Technology, Imperial College, London SW7 2BY. (621)

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†Also post-experience courses not leading to a degree or diploma.

Further details may be obtained from the Academic Registrar, Chelsea College, Manresa Road, London SW3 6LX.

(619)

UNIVERSITY OF STRATHCLYDE
Department of Pharmaceutical Chemistry

MSc Course in Forensic Science

The Department of Pharmaceutical Chemistry, in collaboration with other Departments of the University, offers a 12-month full-time instructional course leading to the University's degree of MSc in Forensic Science. The course embraces a wide range of modern methods of analysis and is specifically orientated towards the requirements of Police, Home Office, and other Laboratories dealing with investigations relating to crime detection, toxicology, etc., and with the presentation of scientific evidence in courts of law.

Minimum entry qualifications: Lower second class honours degree, or equivalent qualification, in Chemistry, Biochemistry, Physiology, Biology, or Pharmacy.

Early application advised for those wishing to be considered for Advanced Course Studentships.

Further details and application forms from Dr. F. Fish, Reader in Forensic Science, The University of Strathclyde, Glasgow G1 1XW.

(594)

CHELSEA COLLEGE, UNIVERSITY OF LONDON
DEPARTMENTS OF PHARMACY AND PHYSIOLOGY
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Tissue Typing: Dr. H. Festenstein.
Immunosuppression: Dr. M. C. Berenbaum.
Allergic Complications of Virus Diseases: Professor C. A. C. Mims.
Auto-immune Diseases: Dr. Deborah Doniach.
Immunological Mechanisms in Allergic Lung Disease: Professor J. Pepys.
Drug Induced Allergy: Dr. J. Brostoff.

Registration forms are available from the Senior Lecturer, Pharmacy Extension Studies, Department of Pharmacy, Chelsea College, University of London, Manresa Road, London SW3 6LX. (614)

UNIVERSITY OF LONDON: A course of two lectures entitled "Algebraic Aspects of a P-Adic Theory of Functions" will be delivered by Professor P. Roquette (Heidelberg) at 5.30 p.m. on February 20 and 22 at King's College (New Theatre), Strand, W.C.2. Admission free, without ticket.—Academic Registrar. (694)

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
DENTAL SCHOOL
M.SC. COURSE IN EXPERIMENTAL ORAL PATHOLOGY

Applications are invited from graduates in the biological sciences for admission to this twelve months' course leading to the M.Sc. degree of the University of London. The course aims to provide a foundation of knowledge of oral disease and in particular of the methods upon which such knowledge is based so as to serve as a starting point from which students might engage in dental or biomedical research leading to a Ph.D. or similar qualification. Students follow a structured course of seminars and practical work for approximately six months after which the majority of time is spent on a research project. M.R.C. training awards may be available for suitable candidates.

The next course starts in October 1974 and interested persons should contact the Department of Oral Pathology as soon as possible, Tel. 01-247 5454, Ext. 89.

Application forms and further information are available from the Dean of Dental Studies, The London Hospital Medical College, Turner Street, London E1 2AD. Closing date for applications is May 31, 1974. (587)

University of Stirling

Technological Economics

M.Sc. and Ph.D. Programmes

Applications are invited for places on the following higher degree courses commencing September 1974:

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Ph.D. Course of three years' duration for graduates in science, engineering, agriculture or economics. Students must complete satisfactorily the assessed M.Sc. course work during their first 8 months (for which they will be awarded a diploma), and then continue with their interdisciplinary research project. The research project studies the interactions between science, technology and economics and is generally associated with the solution of practical problems of resource utilisation in environmental or industrial situations.

Applicants should possess or expect to obtain a good Honours degree or equivalent.

S.R.C. studentships are available for both the courses detailed above.

Further information is available from:

Admissions Officer (PG4),
University of Stirling,
Stirling FK9 4LA,
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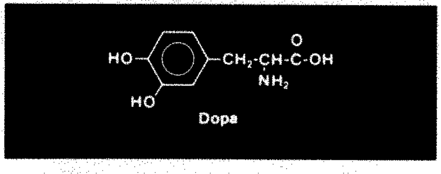

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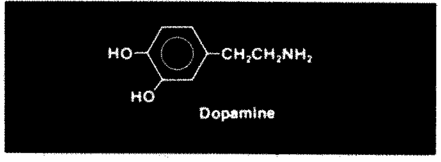

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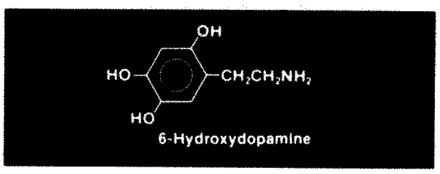

6-Hydroxydopamine

The study of catecholamines, perhaps more than any other pharmacological area,

yields results which rapidly find application in therapy. The treatment of neurologic disorders with **L-dopa** is such a case. A very short time elapsed between the establishment of a role for dopa in central metabolism[1] and its use in the treatment of Parkinsonism.[2] These advances have largely been the result of biochemical studies concerning the conversion of dopa to **dopamine** and the role of dopamine as a neurotransmitter. Thus, dopa decarboxylase inhibitors have been designed to enhance the anti-Parkinsonism action of L-dopa and **α-methyldopa** has recently been shown to be a synergist.[3] Such studies have led to the discovery that dopamine plays the role of a mediator in the discharge of several pituitary hormone releasing factors, increasing growth hormone and decreasing prolactin secretion. The latter observation has resulted in the successful combination of dopa and estrogens in the treatment of breast cancer.[4] Transient effects of dopamine can be prolonged by appropriate derivatization, *e.g.*, amino acid amides of dopamine (we offer the intermediate dibenzyloxydopamine, 16,189-6) are orally effective in greatly increasing renal blood flow.[5] Structural modification of dopamine has led to **6-hydroxydopamine**, a remarkable pharmacological tool for understanding the role and mechanism of neurotransmitters in the sympathetic nervous system.[6] Depletion of catecholamines with this agent is both reversible and irreversible (dose-dependent) and the importance of 6-hydroxydopamine should rival that of reserpine. **5-Hydroxydopamine** also replaces norepinephrine in storage vesicles and its osmiophilic properties enable the replacement to be observed with an electron microscope.[7] The judicial use of enzyme inhibitors and transmitter precursors, *e.g.*, **α-methyltyrosine** to deplete and *threo*-**dihydroxyphenylserine** to selectively increase the concentration of norepinephrine in the brain, will continue to advance the field.

Below is a partial list of catecholamines and amino acid analogs. A complete listing of over 50 enzyme substrates, inhibitors and metabolic products **related to the above** *will be sent on request.*

References:

1) A. Carlsson, M. Lindqvist, T. Magnusson and B. Waldeck, *Science*, **127**, 471 (1958).
2) G. C. Cotzias, M. H. VanWoert and L. M. Schiffer, *New Engl. J. Med.*, **276**, 374 (1967).
3) J. Fermaglich and T. N. Chase, *The Lancet*, 1261 (1972).
4) B. A. Stoll, *The Lancet*, 431 (1972).
5) R. H. Jones, J. H. Biel, W. Ours, I. L. Klundt and R. L. Lenga, 165th National Meeting of the American Chemical Society, Dallas Texas, April 1973, MEDI Abstr. 9.
6) T. Malmfors and H. Thoenen, Eds., "6-Hydroxydopamine and Catecholamine Neurons," North Holland, Amsterdam and Elsevier, N.Y., 1971.
7) J. J. Tranzer and H. Thoenen, *Experientia*, **23**, 473 (1967).

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A journal of exciting biology: Cell

The first issue of **Cell**, a new monthly journal of cell and viral biology from The MIT Press, appeared last month and response has been overwhelmingly enthusiastic. Edited by Benjamin Lewin, formerly the editor of *Nature New Biology*, **Cell** provides a wide-range journal publishing articles of general interest in molecular biology, cell biology, tumor virology and immunology, genetic development and other related areas. Papers are refereed as rapidly as possible and those accepted will be published within twelve weeks.

Cell also includes an extensive review section. In addition to those reviews with the traditional role of discussing a field in some depth, short reviews give conceptual accounts of recent advances in areas of cellular biology, for the benefit of researchers in other areas. Book reviews and discussions of meetings are also included.

The February issue of Cell will include the following articles and reviews:

Viral Hepatitis, the B Antigen and Liver Cancer: A. J. Zuckerman

Integration and Excision of Phage Lambda: M. Gottesman

GUG and UUG are Initiation Codons In Vivo: J. H. Miller

The Molecular Biology of Tumour Viruses, edited by John Tooze: B. Mahy

Altered Metabolism of the Guanosine Tetraphosphate, ppGpp, in Mutants of E. coli: G. Stamminger and R. A. Lazzarini

Continuous Production of Radiation Leukemia Virus in C57BL Thymoma Tissue Culture Lines: Purification of the Leukemogenic Virus: M. Haas

Changes in RNA in Relation to Growth of the Fibroblast. I. Amounts of mRNA, rRNA and tRNA in Resting and Growing cells: L. Johnson, H. Abelson, H. Green and S. Penman

Studies on Amino Acid Control of Cellular Function: V. Morhenn, R. Kram, A. Hershko, and G. M. Tomkins

Translational Capacity of Deadenylated Messenger RNA: E. Bard, D. Efron, A. Marcus, and R. P. Perry

The March issue of Cell will include the following articles and reviews:

Sequence organization of Eucaryotic DNA; defining the unit of gene expression: B. Lewin

Sublines of Mouse 3T3 Cells that Accumulate Lipid: H. Green and O. Kehinde

A Precursor to Hemoglobin mRNA in Nuclei of Immature Duck Red Blood Cells: M. Macnaughton, K. B. Freeman, and J. O. Bishop

Interspersion of Repetitive and Non Repetitive DNA Sequences in the Sea Urchin Genome: D. E. Graham, B. R. Neufeld, E. H. Davidson, and R. J. Britten

A Polymerase Activity forming 5S and pre-4S RNA in Isolated Hela Cell Nuclei: L. McReynolds and S. Penman

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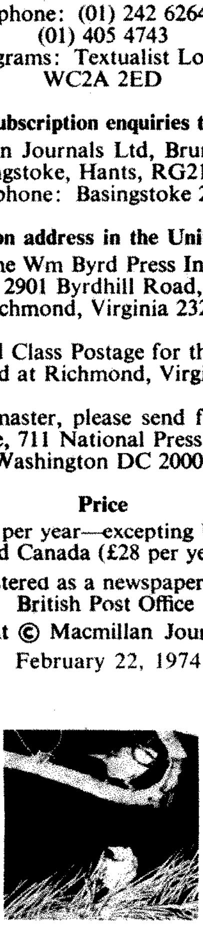

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- 'Matters Arising' permits occasional short discussion of papers that have previously appeared in *Nature*. A limit of 350 words is placed on contributions in this category.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the Système International. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible. exp (a) is preferred to e^a if 'a' is more than one character. Articles should be accompanied by an abstract of not more than fifty words, and the abstract should list the main conclusions that are drawn.

References are indicated by superscripts in the text. The style may be gleaned from any contemporary *Nature* with the following two changes:

(i) If it is necessary to refer to several references by the same author at once, only one reference number need be given.

(ii) The last page as well as the first of any reference should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963–65). 'Personal communication' and 'unpublished work' should be incorporated in the text.

Artwork should be sent with the manuscript. All artwork should be marked with the author's name. Line drawings should preferably be in Indian ink on heavy cartridge paper, although other materials are acceptable; thin, shiny, folded, torn or heavily handled material should be avoided. Matt rather than glossy photographs are preferred. Figures are usually reduced to one column width. The originals should be about twice their final size. Figures, particularly maps, should contain nothing but essential material. It is preferred that the original be unlabelled, but with a copy containing lettering. Labelling on photographs should if possible be avoided entirely. The artwork of published papers can only be returned if a self-addressed envelope is enclosed.

A fuller guide appeared in *Nature* (**246**, 238; 1973).

nature

Volume 247 February 22, 1974

The consequences of Solzhenitsyn

So Solzhenitsyn has been deprived of his citizenship and unceremoniously bundled out of his fatherland in the same way that Lenin was nearly seventy years ago. We have to be grateful, presumably, that the Soviet government did not choose to exact a more loathsome penalty, such as banishment to a work camp in Siberia. We must also be thankful that his family are to be allowed to follow. The fact that worse has not befallen him physically should not, however, diminish our sense of shock that charges of treason should be brought against a man who, from a standpoint of undoubted patriotism, chooses to criticise his country. For if not patriotic (and heaven knows it shines through his novels) why did he not leave his homeland years ago for the comforts of the western world?

He knew that his voice was essentially a Russian one, and he must have known that banishment was the only sanction which could wound his spirit.

The action that the Soviet government took shows the way in which it had allowed itself to become boxed in by one man. In the end, by taking Solzhenitsyn so desperately seriously, it found itself having to use all the state machinery to destroy one single citizen. Yet, at any time in the last year, there was the opporunity to bow out of the conflict and let him make all the running himself. After all, his writings were little known in the Soviet Union, so there were slim prospects that he would be able to gather together any sort of populist following among the Russian public that could ever form a physical threat to internal security: not that he had ever given any indications of a leaning in this direction. Furthermore, there had been sharply divided opinion in the west on the efficacy of his many messages. Many have accepted his observation on the situation in the Soviet Union without too much hesitation, but fewer have been able to go along with his conclusions for political remedy—particularly his calls on western countries to take a tough line in their dealings with the Soviet government.

The way had thus been open simply to isolating and igonring Solzhenitsyn, merely on the grounds that he had a small constituency at home, and was not being effectively harmful to Soviet interests abroad. Instead, the persecution has undoubtedly done this very harm. A most effective way to create a majority is to attempt to destroy a minority by heavy handed means: many who up to the present have been prepared to accept Solzhenitsyn as a great writer but have thought him naive politically are now ready to take him, or rather the political system that he exposes, much more seriously.

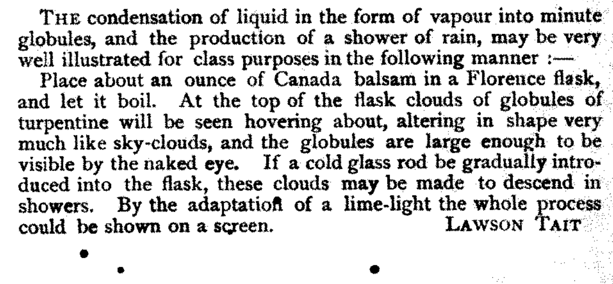
And is Sakharov next?

Once more we shall be swept up in a debate amongst western intellectuals about institutional and individual attitudes to the Soviet Union. If the Solzhenitsyn incident teaches us one thing, it is that ways of thinking in East and West are utterly different on questions of political freedom. It seems that a Hungary, a Czechoslovakia, a Solzhenitsyn is periodically necessary to remind us of this gulf, although, lest we become too arrogant it must be said that there are many calamities attributable to the western political system. Nevertheless, the point is that the difference in character is not something which is likely to be changed by the chilling of diplomatic relationships, by bitterness at Geneva or by the breaking of ties between learned institutions. The official links are all operated by men with seniority and a weight of tradition behind them. These men are unlikely to be susceptible to persuasion.

It is a change in human nature which is being sought by many westerners. Is it foolish and impertinent even to think of wreaking such a change? Probably not. Western attitudes are not what they were ten, a hundred or a thousand years ago, and there is no reason to believe that eastern attitudes cannot change likewise. Indeed, the relative lack of change in recent times makes the potential for future change greater.

This is unlikely to be effected at an institutional level—a parliament or learned body is rarely able to trigger a revolution in patterns of thought. It has to be done by allowing every possible channel of human contact to be exploited. What is needed now is not boycott but more flow, particularly amongst the young.

100 years ago



A Lecture Experiment

THE condensation of liquid in the form of vapour into minute globules, and the production of a shower of rain, may be very well illustrated for class purposes in the following manner :—

Place about an ounce of Canada balsam in a Florence flask, and let it boil. At the top of the flask clouds of globules of turpentine will be seen hovering about, altering in shape very much like sky-clouds, and the globules are large enough to be visible by the naked eye. If a cold glass rod be gradually introduced into the flask, these clouds may be made to descend in showers. By the adaptation of a lime-light the whole process could be shown on a screen.

LAWSON TAIT

From *Nature*, **9**, 323, February 26, 1874.

international news

FDA halts scheme to combat anaemia

Colin Norman, Washington

A CONTROVERSIAL scheme to provide what amounts to preventive medicine through supermarkets and grocery stores has been called off at the last moment by the US Food and Drug Administration (FDA). The scheme involves adding large helpings of iron to most of the bread baked and sold in the United States, in an effort to combat iron deficiency anaemia.

Under pressure from influential medical and nutrition organisations, the FDA issued a regulation in October last year which would have tripled the amount of iron in so-called 'enriched' bread and flour. The regulation, which has the force of law, was due to be brought into effect on April 15, and most bakeries have already made plans to implement it. But a barrage of complaints from individual physicians, concerned about possible danger to people suffering from rare blood disorders, led the FDA to announce last week that it will turn the whole matter over to a public hearing on April 1. The regulation will not be enforced until the safety question has been cleared up, the FDA said.

The idea of adding iron to bread and flour has influential support from the American Medical Association, the Food and Nutrition Board of the National Academy of Sciences, the White House Conference on Nutrition, and virtually every national nutrition association. With such an impressive pedigree, and a goal with which few would disagree, why has the scheme met with so much opposition?

The dispute is, on the surface, simply a straightforward disagreement among scientists and physicians over the interpretation of facts and the results of various clinical and epidemiological studies. But it also touches on some fundamental principles underlying the FDA's approach to regulating the food industry, and it calls into question the federal government's role in trying to improve the nutritional standard of the American diet. In any case, it is an interesting case study of regulatory decision-making.

The idea of fortifying basic foods with iron compounds gained impetus in the 1960s from studies which indicated that anaemia is widespread in the United States, particularly among women and children from low income groups. Since bread is the most widely consumed processed food, it became the obvious candidate for a fortification programme designed to increase iron intake, and in 1969 the idea was floated by the White House Conference on Nutrition as one of its many recommendations for improving the health of the American public.

A year after the White House Conference published its report, the idea was converted into a formal proposal from the American Bakers Association and the Millers National Federation, the baking industry's trade associations. They asked that the legally required amounts of iron in so-called enriched flour and bakery products be increased significantly from their present levels.

The FDA handled the proposal with some caution, first by turning it over to the American Medical Association for advice, and then by coming up with a proposal of its own. It is the FDA's proposal which was issued in final form last October, and which will be the subject of public hearings in April.

In short, the proposal would require that, in order to bear the label 'enriched', all flour sold in the United States must contain 40 mg of iron per pound, and enriched bread must similarly contain 25 mg of iron per pound. According to the FDA, some two thirds of all bakery products now consumed in the United States are enriched. And, in view of the rapid progress of the fortification bandwaggon during the past few years—a phenomenon which has turned most breakfast foods into little more than high-calorie vitamin pills—there is a strong commercial incentive for bakeries to produce goods which can bear the 'enriched' label.

If the proposal is adopted and made a legal requirement, it will not be the first time that basic foods have been loaded with extra nutrients. For many years, vitamin D has been added to milk and iodine has been added to salt, and even iron has, for the past 30 years, been baked into bread. But the proposal represents a radically new policy. The present requirement is that enriched bread should contain between 8 mg and 12.5 mg of iron per pound, simply to ensure that the iron lost during milling of white flour is replaced; it is a policy which is followed in most industrialised countries. The proposed levels, however, represent a deliberate attempt to increase the average American's daily intake of iron.

According to the FDA, even at the proposed levels, the amount of extra iron that would be included in the average diet would amount to less than 20% of the recommended daily requirement. Thus, the agency argued when it published its final regulation, the scheme can hardly be called 'supermarket medication'. The proposal, the FDA said, strikes a balance between providing as much of the recommended daily allowance as possible, and ensuring that the risks to people suffering from blood disorders are kept to a minimum.

But critics of the scheme are not so sanguine. First, they have criticised the studies which led to the theory that anaemia is prevalent in the United States, and they have argued that there is need for more accurate information on the epidemiology of iron deficiency anaemia before such a fortification scheme is begun. Second, they have cited a sheaf of studies which cast doubt on the extent to which iron incorporated in bread is assimilated by man; again, they suggest that more information is needed before iron salts are loaded into bread. And they have also pointed out that nobody has yet managed to quantify the risks of increased iron intake to people suffering from such blood disorders as haemochromatosis, Cooley's anaemia and liver disease associated with alcholism. In other words, even though the idea of fortifying bread with iron was first proposed more than five years ago, the chief argument being put up against the scheme is that it is premature.

As for the prevalence of anaemia, the FDA bases much of its case on a study carried out in the 1960s, called the Ten State Nutrition Survey. This study indicated widespread anaemia, particularly among people from lower income groups, and it also came up with the finding that the problem is not solely confined to women and children. The methods employed in the study have, however, been criticized by several haematologists, who have argued that the conclusions may be false, and also that, even if anaemia is as prevalent as the study indicates, inadequate iron is not the sole cause.

In response to such doubts, the FDA set in train a reappraisal of the literature, from which its experts concluded that "there is a strikingly high incidence of iron deficiency anaemia in many segments of the US population". They also concluded that the studies have "consistently shown higher anaemia prevalence rates among blacks compared to whites, in low income states compared to high income states, and in low socio-economic groups compared with groups higher in this regard". And those conclusions have been endorsed by virtually all the major medical associations in the United States.

But even if the studies are correct, would an iron fortification scheme help? Again, the FDA and the professional medical associations say that it would, and some individual physicians say that such a conclusion is not necessarily supported by the evidence. Writing in *Nutrition Today*, for example, Dr Maxwell Wintrobe, Professor of Medicine at the University of Utah, and a highly respected haematologist, points out that a number of studies have indicated that iron salts added to bread are not readily absorbed by the body. In particular, a study carried out in Wales by Dr. Peter Elwood, which involved two large communities, produced "no conclusive evidence in terms of an effect on circulating haemoglobin levels", Wintrobe asserts. Wintrobe also pointed out that the signs of anaemia picked up by the ten state survey may be put down to a variety of causes, and thus simply increasing dietary iron intake can only solve part of the problem.

The FDA, however, does not concede that the iron fortification scheme would be either unnecessary or ineffective. Dr Alexander Schmidt, Commissioner of the Food and Drug Administration, specifically stated last week when he announced that the proposal would be suspended, that "several tests, including measurements of iron serum, iron absorption, and iron binding capacity have been utilized to verify that low haematocrit and haemoglobin values observed in nutritional studies establishing the prevalence of anemia . . . are due to iron deficiency". Schmidt also cited two studies to show that man does utilize iron furnished by fortified bread. Thus, the FDA has announced that it will not consider further objections on those two points—the only recourse opponents have if the regulation is imposed is to go to court—and the hearings in April will thus be concerned solely with the narrow question of risk.

The hearing will thus be a scientific debate over the likely effects of increased iron intake on people suffering from storage diseases, but two other considerations are likely to arise. The first is that additional dietary iron could mask the diagnosis of diseases which are usually signalled by the onset of anaemia. Cancer of the colon, which causes chronic internal bleeding, is the most frequently cited example. And second, it has recently been suggested that intake of additional quantities of iron could lead to the development of Parkinson's disease in some people, because the disease is associated with increased iron stores in the brain.

The hearings will thus be concerned solely with the risk side of the risk-benefit equation. That is , however, unlikely to satisfy many of the critics of the scheme, who are asking for a full inquiry into the philosophy behind the proposal, and the need for the federal government to require by law that iron be added to bread.

As Dr Wintrobe suggests, if poor nutrition is indeed the cause of iron deficiency anaemia in the United States, a more effective policy should aim at attacking the causes of poor nutrition. Such corrective measures as improving the economic status of lower income groups, reducing unemployment and lowering food prices would be more effective than "to cover our eyes, shut our minds, triple the dietary iron and hope for the best", he says.

Super bread and frozen hay

from our Soviet Correspondent

Two new nutritional advances have been announced by Soviet Scientists. A team working at Tashkent, in the cotton-growing Uzbek SSR has developed a method of extracting albumin from cotton seeds. The product, when added to dough, is said to result in bread identical in taste to the ordinary kind, but with a nutritional value equal to that of meat.

Meanwhile, down on the collective farm, an improved winter fodder is planned for livestock. Instead of the old-fashioned uneconomical hay, which loses between 20 and 40% of its nutritional value in the drying process, a new system of instant deep freezing of the mown grass at −17°C promises an all-year-round diet of fresh grass, with a carotene content twice that of hay.

British MPs do not like US reactor plan

John Hall

THE House of Commons Select Committee on Science and Technology, which has been examining the possible choices of nuclear reactor systems for Britain, has rushed out a report in record time in order not to be presented with a *fait accompli* by the government (Paper 145, HMSO., 1974; 14p). In the event Parliament was dissolved before any decision could be taken but the committee was nevertheless justifiably proud of its fast move. Its recommendation, which was pretty much a foregone conclusion, is that no proposal to build American light water reactors under licence should be approved on the basis of existing evidence. Questions of safety, costs and the effects of a purchase from the United States on Britain's own nuclear development programme are the bases of the committee's opinion.

To order light water reactors (LWRs) in the numbers proposed by the Central Electricity Generating Board (CEGB) would mean (the committee thinks) that apart from work on the fast breeder reactor Britain would virtually be abandoning its long established nuclear research and development effort.

As for the safety angle, the committee considers that in view of the conflicting opinion on the reliability of LWRs, it is for the proponents of light water technology to prove its safety beyond reasonable doubt, rather than for their opponents to prove the contrary case. The point is of particular importance for a densely populated country like Britain, it says.

Evidence the committee heard on costing was so varied that members grew to be sceptical of any hard and fast quotation, regardless of its source. The report comments that no part of the evidence received on capital costs is directly comparable with any other part; this leads the committee to suspect that, unless there is a great deal of operating experience with the system in question, no one could guarantee that any given reactor system would prove cheaper than any other under actual operating conditions. In its opinion no sufficiently large nuclear reactor system has been operating for long enough to permit such confident estimates of installation and operating costs as those which the CEGB put forward to justify its preference for LWRs.

Common sense indicates, says the report, that until the high temperature gas cooled reactor (HTR) and the fast breeder reactor are available on a commercial basis the way forward should be to use one of the British nuclear technologies which is already proven, since this would be likely to satisfy the Nuclear Inspectorate without undue delay. Bearing this kind of consideration in mind, the committee notes in its report the enthusiasm of the South of Scotland Electricity Board (SSEB) for the steam generating heavy water reactor (SGHWR).

The findings make the CEGB preference for American technology look distinctly shaky, since the mainstay of this preference is the cheapness and ready availability of the LWRs. Since the committee found costing estimates so wildly varied as to be unreliable, and the Nuclear Inspector's likely requirements so time consuming, the CEGB is left with the onus of offering better supported cash estimates, specifications less suspect to the Nuclear Inspectorate or an entirely new set of justifications for the hardware. At this stage of the game this does not look like a good exercise to be starting.

The CEGB had wanted to order nine twin-reactor stations, each of capacity 2,400–2,600 MW, between now and 1979, regardless of the choice of reactor. Mr A. E. Hawkins, Chairman of the CEGB, although denying that all of these orders would be for pressurised water reactors (PWRs) of American design, gave evidence to the committee which nevertheless suggests that this reactor would in fact account for the bulk of the CEGB's new nuclear plant if his plans were accepted. He had admitted, in the face of questioning, that LWRs of 1,200 to 1,300 MW are not yet in service, and therefore not proved.

The report comments that, since calculations up to 1980 are still being made on the basis of an annual growth in load of 4¾%, the committee is at a loss to see a pressing need for a decision to implement a programme of this size over such a long time scale, particularly as Mr Hawkins had said that the CEGB were, even before the current crisis, "very much exercised about the energy availability for the production of electricity in the 1980s and 1990s".

Unfortunately for the CEGB, Mr Hawkins suggested in 1972 that there was no great urgency about the choice of reactor system, and basing his calculations on a load growth of 5%, he said it was probable that only four new nuclear reactors would be required before 1982. Since these views were evidently mistaken, the committee remarks acidly, it will need much greater assurance that the CEGB's new plans are based on more valid assumptions.

Forward planning estimates and cost considerations apart, the American equipment simply will not come within a hundred miles of the English countryside if there is a really strong body of opinion that one day it may just give out and spill its contents, and the decision of the Nuclear Inspector is crucial to this issue. The report notes that although proponents of the PWR are convinced of its safety the Americans themselves are sufficiently doubtful to be building a small PWR somewhere in the Idaho desert, where it is to be tested "almost to destruction" in order to determine whether or not it deserves a reputation for safety.

Reinforcing the doubt in this area, the report gives a prominent reminder of a memorandum submitted by Sir Alan Cottrell, the Chief Scientific Adviser to the Government, and one of Britain's most eminent metallurgists. Sir Alan's evidence read:

"Rapid fracture, from large cracks or defects in thick sections, is in principle possible in steel pressure vessels under operational conditions. In LWR vessels the estimated critical crack size for unstable growth is smaller than the wall thickness, so that the 'leak-before-break' safety feature is unavailable. In these circumstances, the security of an LWR vessel against fracture depends on the maintenance of rigorous manufacturing and quality control standards; and on thorough, effective and regularly repeated examination of the vessel by the ultrasonic crack-detection technique. The possible gradual growth of small cracks in highly stressed regions, by ageing and corrosion effects during service, needs further scientific investigation; as also does the effect of thermal shock from emergency cooling water in a loss-of-coolant incident."

The safety issue is all the more important since operating experience with PWRs of 500 MW and above is still very limited. Mr F. L. Tombs, Chairman of the SSEB, gave an estimate of about 20 reactor years of experience for large PWRs as against 200 reactor years of experience for British Magnox plant, and as recently as November Commissioner Doub of the United States Atomic Energy Commission was reported as saying that "while the technical feasibility and demonstration of the technology is a quarter of a century old the operating experience, especially with nuclear plants of the 800–1,000 MWe variety is still quite minimal".

The committee nicely sums up the impossible nature of the calculations involved in one distinctly world weary paragraph. In addition to the possibility of redesign to meet British safety requirements, they note any argument on the economics of reactor systems is influenced by variables including those in the following list:

"The sizes of the plants which are being compared, the dates to which the quotations refer, the estimates of availability and load factor for the systems in question, the extent to which wage escalation during construction and rises in fuel costs are taken into account, and the length of time which it takes from the first decision to purchase a station to its entering commercial operation." There is no actual mention of Gordian knots or Augean stables, but there is more than a suggestion that that kind of job pales into insignificance.

Appointments down under

from a Correspondent

Two Americans have been appointed directors of the two largest, new research institutes in Australia. The Australian Institute of Marine Science (AIMS) will be headed by Professor Malvern Gilmartin, at present Professor of Biological Oceanography at the Hopkins Marine Station of Stanford University, and the Anglo-Australian Telescope (AAT) will be directed by Professor E. Joseph Wampler. The two will take up their duties later this year.

Four and a half years have elapsed from the initial announcement of the formation of AIMS by the previous Liberal-Country Party government of Australia. Negotiations over the site for AIMS between the Federal Labor Party government and the Country Party government of the State of Queensland have just been concluded with the designation of a site of 50 acres at Cape Cleveland, near Townsville in North Queensland.

Professor Gilmartin, whose advertised salary of $A 19,148 is under review (it is at present the same as that for university professors), will have $A 8 million to spend in the first five years. The institute is charged with researching the little known tropical and semi-tropical waters of Australia, notably in the regions of the Great Barrier Reef, the Coral Sea and the North Queensland coast.

The AAT is a 3.9 M (154-inch) optical reflector being built jointly by Britian and Australia. Professor Wampler's directorship is "for two years in the first instance", during which time he will be on leave from the Lick Observatory of the University of California where he has been an instrumental astronomer since 1967 with a reputation for playing a leading part in developing the image intensifier system.

The AAT is a highly instrumented and automated piece of equipment. It will probably turn out to be fractionally larger than the other big reflector in the southern hemisphere (3.9m), being built at the Cerro Tololo Observatory in Chile. The AAT is now in the final stages of construction on Siding Spring Mountain in north-western New South Wales. The commissioning astronomer, Professor S. C. Ben Gascoigne, of the Australian National University, is already integrating and calibrating all the Telescope's systems: he has expressed his enthusiasm for the high quality of the mirror which arrived recently from England.

The AAT will be fully operational by January 1975. The cost of establishing the AAT by then will have been $A 16 million, shared equally between the United Kingdom and Australia. The AAT board has recently announced its estimates of the running costs: these will rise to $A 1.74 million per annum in 1976-77 before steadying out at $A 1.3 million per annum in following years. Professor Wampler's salary has not been announced.

In Australian scientific circles, there is much relief that these two projects in growth areas of Australian research can move into high gear at last, though there has been disappointment among local scientists that none of their number won either of these key posts.

The temporary nature of Professor Wampler's initial appointment underscores the uncertainty which has hung over the running of the AAT, as distinct from its design and construction which proceeded smoothly. There was much heartache over whether the Australian National University would have any special place in running the astronomical programme of the largest telescope on the site of their own Sliding Spring Observatory: the university lost on this point. The head of its Department of Astronomy, Professor Olin Eggen resigned from the AAT board last year. After protracted discussions, the AAT board, with equal representation of three Australian and three British nominees, did not settle on one of their own world class nationals as director. But, at least the AAT board has determined a policy of observing time being equally divided between British and Australian astronomers—under a tactful director of neutral nationality.

Oxfam solves a sewage problem

John Wilson

In the hope of cutting down on infections caused by insanitary conditions in refugee camps Oxfam, the famine relief organisation, has commissioned original scientific research and is well on the way to developing an entirely novel kind of sewage disposal. Already it has arrived at a system which scores over conventional equipment by being cheap, easily transported, and capable of erection by unskilled labour. It may also result in the elimination of the organisms responsible for cholera and dysentry by containing sewage in anaerobic conditions.

Oxfam's interest in a sewage system was first kindled by its experience of the refugee camps in Bengal at the time of the Indo-Pakistan war of 1971. Oxfam realised that most of its relief effort was often spent on the treatment of diseases resulting from insanitary conditions within the camps and that in many instances the preventive medicine used was not very effective in controlling these diseases—particularly cholera.

Containment of the sewage is a priority, so Oxfam looked for a suitable container. It considered using a portable fuel tank, developed by the Royal Air Force (RAF) as part of its back-up facilities for the harrier, and found that it would provide almost instantaneous anaerobic conditions. From this sprang the idea of destroying the pathogens anaerobically.

In the event, Oxfam found that the RAF tank had some shortcomings and decided instead to pass the effluent along a flexible tube about 100 feet long and 5 feet in diameter. This can be rolled up for transport and easily partitioned for the sedimentation of solid matter. Moreover, by sampling the effluent as it moves along the tube a ready estimate of the survival of pathogens can be made.

The water pollution research laboratory at Stevenage advised on the general design of a unit suitable for about 5,000 people, but there was one important fact that the Stevenage laboratory did not know. The survival time of the cholera vibrio in anaerobic sewage had never been determined, so it was not clear how long the sewage would have to be retained to eliminate the organism. Oxfam asked Mr B. Lloyd, of the University of Surrey, to study the retention problem.

With the assistance of a student, Mr Lloyd found that the survival of the organism depends on the temperature of the sludge. No cholera vibrios were found when the effluent was maintained at 37° C for 7 days but when the temperature was lowered to 25° C they took 12 days to disappear. In his final report to Oxfam, Mr Lloyd recommended that the sewage should be retained in anaerobic conditions for at least a fortnight. This piece of research cost Oxfam £50.

With a grant of £19,000 from the Leverhulme Trust, Oxfam next set about trying the system under operational conditions. They sent a microbiologist and two engineers to Bangladesh at the beginning of the year and on the evidence of their visit it is likely that there will be a large scale test project at a Bihari refugee camp in Muhamadpur, Dacca, in the near future. An extra £6,000 is needed before the plan can go ahead, but there is a suggestion that the government may come forward with the money. With the evidence of such enterprise on the part of a registered charity, it can scarcely do otherwise.

Setback for space science

Colin Norman, Washington

A LAUNCH vehicle designed to be NASA's chief workhorse for planetary flights in the 1970s, had to be blown up during its test flight last week. The rocket, a Titan-Centaur, was destroyed over the Atlantic when the Centaur stage failed to ignite. A board of inquiry has been set up to try to determine the cause of the failure, but from preliminary data, it seems that a boost pump failed to supply necessary pressure to the Centaur engines.

The episode has caused some consternation among planetary scientists, because the Titan-Centaur is designed to launch six important missions in the next few years—two Helios spacecraft to study the Sun, two Viking missions to Mars, and two Mariner spacecraft on a flyby mission around Jupiter and Saturn. If the failure is deemed serious enough to require a second test mission before the launch vehicle is entrusted with an expensive spacecraft, some of those missions may have to be delayed.

NASA officials last week were hopeful, however, that a second test flight will not be necessary, and that the first Helios launch can take place on schedule in September. According to Mr Joseph Mahon, director of NASA's launch vehicle and propulsion programmes, the flight provided much valuable information as far as it went, and many pieces of hardware specifically under test functioned perfectly. The guidance system, for example, which is located in the Centaur stage took the Titan stage on the correct course, and the shroud—the only major piece of equipment that had not been flown before on other rockets—jettisoned successfully.

The flight also provided data on the environment in the spacecraft section as it went through the lower reaches of the atmosphere, which is essential for the planning of future missions. Thus Mahon predicted that if the board of inquiry can pinpoint the failure exactly, then "we can launch Helios with a 99.9% certainty that it will function properly".

The failure itself occurred some eight minutes into the flight, as the vehicle was passing out of range of the Kennedy Space Center's tracking equipment. Thus it was not possible to tell with any certainty exactly what went wrong until data were relayed back from tracking stations further down line.

Apart from the Centaur rocket itself, its payload, consisting of a model of the Viking lander, and a spacecraft designed to measure the effect of charged particles on solar cells, was also blown to pieces.

Christmas prize quiz solution

The best answer to the *Nature* Christmas Prize Quiz (**246**, 385; 1973) came from Nicholas Kennedy of the Max-Planck-Institut Für Molekulare Genetik, who wins the prize of a year's subscription to *Nature*.

The correct solution is as follows, with Kennedy's answers in parentheses where appropriate:

1 High flux reactor: France and Germany. **2** The White House. **3** Zhores Medvedev. **4** Lynden-Bell and Hoyle, now at Manchester. **5** Glaciation on Mars. **6** Advisory board for the research councils. **7** Computer printout from an aggressive game developed by Maynard-Smith and Price. **8** Britain and the Netherlands. **9** Appointed Chairman (*sic*) of Atomic Energy Commission. **10** Michael Heseltine misled the house about Hovertrain's fate. **11** Lord Snow (John Maddox). **12** Always. **13** J. Watson speaking of cuts in research funds. **14** six. **15** Sir Arnold Weinstock. **16** Japan. **17** Heimaey. **18** Hovertrain scrapped. **19** Motor car manufacturers (but not people who breathe air). **20** From $Z = 2.9$ to $Z = 3.4$. **21** 5.9×10^6. **22** Sakharov. **23** 2030 AD. **24** Six and three. **25** S.F. Edwards. **26** Dinosaurs. **27** 747 members of the Royal Society (the Elephant and Castle Bingo Club). **28** Stones, Tree trunks and large rocks. **29** Aigrain, Paris, MIT. **30** A Council for Science and Society. **31** Intraplate earthquakes. **32** Allotting money for sortie-lab. **33** Commité Recherche Européuene Science et Technologie: selling price increase tax (Society for the Prevention of Inane Tests). **34** International Union of Pure and Applied Physics: Voronel was not allowed to attend meetings in Amsterdam and Moscow. **35** Letcombe Laboratory. **36** 2.9 M.Y. **38** Margaret rejoined Geoffrey in California. **39** Cottrell. **40** January 2 to 5, 1974.

An innocent abroad: science in London

John Gribbin

Just how badly is the present series of crises affecting that most important of scientific traditions, the symposium or seminar? Without the cross pollination provided by these meetings, science as we know it would be impossible; learned societies are still managing to meet regularly, but not without difficulties, as I discovered on a recent visit to the Scientific Societies Lecture Theatre at Savile Row in London. To be fair, all the happenings of that eventful afternoon are unlikely to coincide often—but they do provide a potted guide to the hazards of attending scientific meetings in February 1974.

Everything began well, and it was a relief to leave the candle-lit *Nature* offices, even if only for the slightly less unrelieved gloom of the tube. The meeting itself was a Specialist Discussion of the Royal Astronomical Society (RAS), on "Extragalactic Radio Sources", and began on time in the cosy lecture theatre at Savile Row. Unfortunately, however, these meetings are beginning to suffer somewhat from the problems raised by their own success. They were introduced relatively recently, in an attempt to break down some of the traditional barriers of formality at the monthly meetings of the RAS and to provide a forum for more informal discussion of specialist topics, where "A Fellow" could stand a chance of contributing something worthwhile to the proceedings.

The first series of these new meetings was a huge success, in spite of the cramped conditions at Burlington House where they were then held, and rooms were literally packed to overflowing with fellows eager to keep up with advances being made at the frontiers of astronomy. Now everyone can sit in comfort in the luxurious accommodation at Savile Row. But the speakers are removed from the audience by the usual paraphernalia of lecterns and so on, and the seats both encourage drowsiness and make Fellows reluctant to stand up and be counted. The result is that the meetings, instead of providing a lively forum, are now no more than a series of highly informed lectures, which are certainly among the best of their kind, on occasion, but which are drifting back into the rigid chairman/speaker/audience format of old.

Indeed, I confess that, presented with what seemed to be a choice of a quiet snooze or getting an early train home, I succumbed to the latter temptation and left the proceedings early. That is when my troubles began.

The nearest tube station to the lecture rooms is Oxford Circus. Progress in that direction was impeded by what seemed to be half the British student population, filling Oxford Street while demonstrating in favour of higher grants, and chanting "Heath out," a suitably apposite slogan on the day Parliament was being dissolved. But it was possible to squeeze into the station and get as far as the platform, where a loudspeaker was busily requesting passengers to leave at once because of a bomb scare. Nobody seemed too worried about this probable hoax, except two schoolgirls who ran squealing up the escalator as if they expected to meet Donny Osmond at the top.

So it was back into the fray above, and a brisk walk down Regent Street to Piccadilly Circus, where there was no bomb scare, but the journey now involved a change at Green Park underground station in order to get to Victoria. At Green Park there was a remarkable sight—a genuine practitioner of the three card trick, actually persuading mugs to deposit money with him. The only surprising thing about the three card trick is that anyone can believe it is not fixed, but in front of an admiring audience this practioner took two customers for a pound each, while the nearby busker, striving to earn a reasonably honest living by playing what seemed to be a bass oboe, was passed by on the other side. Perhaps there is a moral to be drawn here.

Resisting the temptation to invest £1 (it looked so easy) I hurried on to Victoria and points south. Or so I thought. A blank departure board (the biggest of its kind in the world, we are informed) told the sad story. No trains to Sussex, let alone Brighton. An appropriate end to a curious afternoon.

Push for solar power in the United States

The US House of Representatives last week passed a bill which would provide some $50 million over the next five years in government money to subsidise the development of solar heating and cooling devices. The bill, sponsored by Mike McCormack, a Democrat from Washington State, was passed by 248 votes to 2, a margin which indicates that in election year the energy crisis is good for voter appeal.

The idea is to add money to NASA's budget to enable the space agency to let contracts with industry for fabrication of residential heating and cooling devices. The goal is to develop 4,000 units in the five-year period. Half the units would be installed in federal buildings or federally owned houses. The other half of the units would be installed in private houses, at no cost to the owner, but they would remain the property of the US government for five years so that their performance can be evaluated.

McCormack pointed out during debate on the bill that if the programme is successful in persuading 5% of the houses and buildings in the United States to generate 80% of their heating and cooling requirements from solar energy, the bill will save 600,000 barrels of oil a year by the mid 1980s.

The bill now goes to the Senate, where prospects for its passage are bright.

Thus, with $50 million proposed in the Administration's budget for research and development on solar energy, and McCormack's bill providing another $50 million over the next five years to exploit the technology, those who have been advocating this energy source for years must be delighted by the advent of the energy crisis.

The only cloud that slightly dims the outlook for this bill in particular, and for the solar energy programme in general, is that there may be a fight between Congress and the Administration over who should have charge of the effort. It became clear during debate on the bill that the Administration, acting through the Office of Management and Budget, suggested to several Congressmen that NASA is not the best home for such a programme, and that the bill should be delayed until more permanent arrangements have been worked out for the management of the federal government's entire energy research and development programme. Such an arrangement—the Energy Research and Development Administration (ERDA)—has already been approved by the House of Representatives, but may become becalmed in the Senate. In the event, however, the House decided to give the authority to NASA, but to transfer the programme to ERDA if, and when, it is established. It remains to be seen whether the Administration's opposition will have any effect on the Senate's consideration of the bill, but electoral appeal is likely to outweigh White House pressure.

Exciting end to British Chess Championship

Jonathan Penrose

THE British Chess Championship was first held in 1904 and since that time, with one or two breaks, has been an annual summer event lasting about a fortnight. The result of the 1973 championship held at Eastbourne ended in a tie between Michael Basman and William Hartston (both in their mid-twenties). There was no time for an immediate play-off for first prize so that the issue hung in the balance until the week of January 28-February 3, 1974 when a six-game match was duly played in the luxurious ballroom of the Heathrow Hotel.

The styles of the two players were sharply contrasted. Hartston has a sound knowledge of theoretical lines of play (based on systematic homework of opening developments in international play) and favours a logical plan of campaign, whereas Basman is a tactician who spurns orthodoxy and seeks to create unusual positions even in the earliest stages of the game. The course of the match (a 4½–1½ win for Hartston) tended to illustrate how hard it is becoming to be truly original in chess and at the same time maintain a basic soundness in strategy. Hartston's first British Championship win was, however, well merited.

Other matches taking place early this year are those of the quarter-finals of the 'candidates' matches. These involve a series of knock-out matches to decide who is to challenge Fischer for the World Championship scheduled for 1975. Boris Spassky (the defeated champion in 1972) seems to have regained his form, having recently won the Soviet Championship, and he convincingly defeated Robert Byrne (United States) in one of the candidates matches. His opponent in the semi-finals will be the young Soviet player Anatoly Karpov, who is hailed as a future world champion. The match between these two should be hard-fought and close. The other half of the draw for the candidates matches also seems likely to be dominated by Soviet players with Petrosyan and Korchnoy the most likely protagonists for the semi-finals.

In the shadow of these candidates matches lies the enigma of the role that Fischer is prepared to play in them. He has not played a single serious game since his match with Spassky in 1972 and whether or not he will play the winner of the candidates matches must still be considered an open question. All chess enthusiasts must surely hope that he will play.

James Hart

SCIENTISTS at the Royal Aircraft Establishment at Farnborough agree on a show of hands to take industrial action in support of their wages claim. The Farnborough men were among the 30,000 scientific workers whom the Institution of Professional Civil Servants (IPSC) was preparing for one-day strikes and half-day walkouts in order to stir up some enthusiasm for their claim in government circles. The dispute threatened to affect 16,000 Civil Service scientists and something like 15,000 related workers in the United Kingdom Atomic Energy Authority, the Research Councils and the Veterinary Service, as well as lecturers in defence colleges, patent examiners and even the Meteorological Office's television weather men. Thousands of members took part in half-day strikes or 'non-cooperative' working at establishments throughout the country and, after a week's respite called by the union to let the government offer an interim increase or the Pay Board an early report, the scientists warned that they could escalate their action in order to hit at 'sensitive points' relying on IPCS scientists. The dispute was caused by a disparity between the pay of scientists and that of comparable grades in the executive branch of the Civil Service. The scientists have been trying to get their pay structure re-assessed since 1971 and expected a settlement in January. Instead, they were given the news that the Pay Board was unlikely to report before March at the earliest. The General Secretary of the IPCS, Mr William McCall, says the dispute is a story of a reasonable group of workers, opposed to militancy but driven to it by bitterness and anger. It is, in his view, a case bearing the hallmarks of the exploitation by a government of the industrially weak.

correspondence

Tank mentality

SIR—It is disappointing to find the editor of *Nature* advocating the use of think tanks (*Nature*, **247**, 169; 1974). A tank is conceptually a rather isolated system. It has inflow and outflow pipes and, hopefully, check valves but it does not otherwise interact with the outside world. Interaction, however, as Robert Eisler used to emphasise, is the basis of our sense of reality. It requires a tank mentality to lift the customer-contractor principle from one field, trade and manufacture, where it is appropriate, to another, research and development, where it is not. A customer may reasonably contract to purchase a specifiable product such as a type of refrigerator, the performance and production costs of which are known. In contrast, it is a grave risk (outside the tank world) to make a contract for some system which has not previously existed, in the development of which materials and systems are to be used in a new way. What but a tank mentality could give rise to phenomena like the notorious groundnuts scheme, could accept 'estimates' for the costs of certain Rolls-Royce engines or for Concorde or could envisage estimating the cost of producing a new crop variety in agriculture with specified yield and characteristics of resistance to disease.

The conflict between planning or drawing office, which produces a design, and the shop floor, which finds it impractical, is too common. As the scale of human planning increases, the drawing office becomes larger and more distant from operational reality. Planner and producer become more widely separated; more impractical, damaging or disastrous schemes are floated. What is needed is a thinker-doer mentality. Planners should leave their offices to participate in the execution of their plans to return as wiser men taught and stimulated by their shop floor or field experience.

It will be protested that some can think but cannot do and are better in the planning office, whereas others can do but cannot think and are better on the shop floor. True, but neither of these groups should be given responsibility beyond its limited experience for evaluating or developing new schemes. Positive steps are needed to provide a supply of citizens experienced and capable in both activities, thinking and doing.

Science has shown great success at prediction from general principles but if *hubris* or just crass ignorance should lead us to underestimate the complexity of the world about us, we shall fall. Let us not forget the advice of Columella, author of that fascinating encyclopaedia of agricultural experience, the *De Re Rustica*, written in the first century of our era (I quote from the Loeb Classical Library edition, 1960, Book I, 1, 16):

"*Usus et experientia dominantur in artibus, neque est ulla disciplina, in qua non peccando discatur.*"

"It is practice and experience that hold supremacy in the crafts, and there is no branch of learning in which one is not taught by one's own mistakes."

Yours faithfully,

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ERTS

SIR,—Due to postal delays I have only recently been able to read your editorial "ERTS—Technological Success, Scientific Failure?" (*Nature*, **245**, 345; 1973). To me it was a thoughtful questioning of a programme involving a combination of science, technology, and bureaucracy.

I have had a bit of experience with geologic interpretation of space imagery, which has a distinct advantage when one wants to step back for a regional perspective of major structural trends that cannot at present be viewed in other ways. Mosaics of aerial photographs are almost always uneven in quality, the unevenness greatly inhibiting a regional analysis. Enough has been said about the necessity of ground-truth (confirming one's interpretation of imagery data by checking features on the ground) for one to appreciate the work and expense involved in analysing ERTS imagery. It is worth emphasising (as you did) that the resolution is only sufficient for viewing large features, although, as regional geologic trends can often be viewed as large features ERTS is still quite useful here. In other words, aerial and space imagery are not mutually competitive, but are complementary.

You also rightly note that weather and constant sun angle conditions mask many areas as well as many features within a given area. But weather conditions are as much a problem for conventional aerial surveys in areas such as tropical, developing Ghana. Here a Canadian team has been stationed for a couple of years trying to conduct a survey that is usually hampered by cloud cover during the rainy season, and thick dust cover for much of the dry season. Nevertheless, the crew must stay here to be prepared to fly on that rare day when conditions are right. Similar climatic hindrances exist over much of the earth's surface; and I am sure that quite a lot of money is similarly spent on conventional aerial surveys elsewhere with only meagre results during long periods, unless one is satisfied with only radar imagery (which is better than nothing, but has considerable limitations). ERTS may not be especially expensive in this regard at least in operating costs, when scale of coverage is considered.

As an earth scientist I feel that ERTS must be tested for some time, eventually proving itself a scientific, technological, and economic success or failure. What worries me is that the agencies involved, such as NASA and the United States Geological Survey's Earth Resources Observation Systems (EROS) programme might, for short term prestige purposes prematurely push to prove ERTS by spending on expansion before such expansion is justified. There certainly are indications of overeagerness to declare ERTS a success, and to rapidly expand the programme. For example, about three years ago, when EROS was pushing for—and receiving—large budget increases, I saw a promotional brochure proclaiming that "automated snow mapping" had been accomplished. Having done field work in their study area I had a good chilly laugh noting that their claimed delineation between "thick" and "thin" snow cover was actually a map of the tree line! In their haste to "prove" a point, they must have failed to make that essential ground-truth check.

Though I disagree with you in some details, my general opinion is similar. NASA shouldn't consider ERTS-B until scientists and technologists outside the sponsoring agencies have concluded that a successor to ERTS-A would be worthwhile, and that at least part of the additional sea of data would be analysed. But ERTS-B should not be forgotten, only relegated to the subconscious.

Yours faithfully,

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news and views

Homology and structure of transfer RNA

MUCH interest at present centres on initiator transfer RNA which in its aminoacylated form donates a methionine residue to the N-terminal position of nascent proteins in the initiation of protein synthesis. These $tRNA_f^{Met}$ species are distinguished from other methionine specific tRNAs in that they can only participate in initiation and not in elongation; this second role is reserved for $tRNA_m^{Met}$. All the initiator species have several common biochemical properties in that they can all act as substrates for *Escherichia coli* methionyl-tRNA synthetase and for *E. coli* methionyl-tRNA transformylase. On the other hand initiators from prokaryotes and cell organelles are distinguished from those from eukaryotic cytoplasm in that the latter are not formylated *in vivo*. There is also some evidence that the course of initiation in mammalian cytoplasm proceeds in a different manner than in prokaryotes; thus the binding of $tRNA_f^{Met}$ to the 40S ribosomal subunit in mammals is independent of template suggesting perhaps that the tRNA bound to the ribosome selects the initiation region of the mRNA rather than the reverse.

It is to be expected that these differences and similarities in the behaviour of initiator tRNA species is reflected in their three-dimensional structures and hence in their sequences. Research by two groups of workers reported on pages 516 and 518 of this issue of *Nature* suggest that this is the case.

Piper and Clark describe the complete sequence of cytoplasmic $tRNA_f^{Met}$ from mouse P3 myeloma cells and Simsek, RajBhandary, Boisnard and Petrissant give that for rabbit liver cytoplasmic initiator tRNA. It turns out that these two sequences are identical. Moreover, Simsek *et al.* provide strong evidence based on the identity of olignucleotide fingerprints that the initiator from sheep mammary gland cell cytoplasm is also the same. It would thus seem that all mammalian initiators are identical. Although this identity does not extend to all initiator molecules, comparison with other published sequences shows extensive homologies between the mammalian sequence and that from yeast; indeed, notable homologies even exist with *E. coli* $tRNA_f^{Met}$. No doubt these homologies are related to the biochemical similarities between the initiators already noted, as well as their special role in protein synthesis.

On the other hand, the prokaryotic and eukaryotic initiators are sharply distinguished in that the latter—in mammals and yeast—both possess in loop IV of the clover leaf structure the unique sequence A-U-C-G-m^{1}A-A-A whereas *E. coli* has the sequence T-Ψ-C-A-A-U with the conventional T-Ψ-C triplet and a pyrimidine in the last position. Unpublished work quoted by Simsek *et al.* shows that plant cytoplasm initiator also has A-U*-C-G-m^1-A-A sequence with a modified U so that the odd sequence seems to be universal in eukaryotic cytoplasmic initiators.

Another feature of interest in the mammalian sequence is the presence of a C residue immediately preceeding the anticodon instead of U which is otherwise universal. This feature is absent in yeast and its significance remains unclear.

Piper and Clark quote unpublished work to the effect that the two isoaccepting $tRNA_m^{Met}$ species from mouse myeloma cytoplasm contain conventional sequences in loop IV. These molecules are not formylated but they do act as substrates for *E. coli* methionyl-tRNA synthetase. These observations strengthen the notion that the unconventional sequence in the eukaryotic initiators is related to their peculiar role in protein synthesis. It is to be presumed that this relation is mediated through alterations in the three-dimensional structure of loop IV but at present there is no evidence on this point.

The question of the secondary structure of tRNA has been taken up by Kearns, Wong, Hawkins and Chang in a communication in the same issue of *Nature* (page 541). These authors are concerned with the difference between the secondary structures of native and denatured tRNA as manifested particularly by yeast $tRNA_3^{Leu}$ and they have compared the high resolution nuclear magnetic resonance (NMR) signals obtained from these two stable conformers. Previous work had established that each Watson-Crick base pair produces a single resonance in the low field (11 – 15 p.p.m.) region and analysis of the low field spectrum of the denatured and native forms of $tRNA_3^{Leu}$ revealed that the first has 18 ± 2 base pairs and the second about 4 more. The detailed analysis

Oil and Earthquakes

A REMARKABLE and exciting set of observations is reported by Arieh and Merzer on page 534 of this issue of *Nature*. Oil-field technology and earthquake prediction are brought to each other's aid by means of an astute comparison.

For several years the oilflow from wells in the Gulf of Suez has been subject to periods of large fluctuations interspersed between times of relative uniformity. These periods have correlated across a set of several wells. Oil is forced out of the wells by the hydrostatic pressure of the overlying rocks and so fluctuations in flow are presumably caused by variations in the pressure at depth. The weight of the rocks clearly does not change so what are observed must be variations in the way that this pressure is transmitted to the interstitial fluid. That is, effects of varying pore pressure are being seen. Arieh and Merzer observe that the times of maximum fluctuation precede the occasions of large earthquakes a hundred kilometres away at the north end of the Red Sea. The quakes seem to restore calm to the oil flow. Their first figure tells the whole story.

The observations cannot but strengthen the seismologist's belief that some earthquakes are predictable by precursory dilatancy changes in the rocks in the vicinity of the focal region. The time duration of the effect is in good agreement with previous approximate relationships between precursor time and magnitude. Moreover, the observations demonstrate that the dilated region is large, also in agreement with other work that has been published during the past year.

Oil companies and earthquake geophysicists have a lot to tell each other in the months ahead.

D.D.

of the spectra also showed that the difference between the two forms is not merely that the D form has lost a few base pairs. Instead, so the data imply, at least five pairs are lost from the N structure and two reformed in the denaturation reaction. Thus the secondary structures of the two forms are different and not merely variants produced by a small unfolding of the clover leaf structure. Semi-empirical rules already available allow the calculation of the NMR spectrum corresponding to a given secondary structure and there is good agreement between the observed spectrum of the N form and that calculated for the conventional clover leaf model.

Kearns *et al.* investigated several other alternative structures and compared their calculated spectra with that observed for the D form. All the models tried retained the amino acid acceptor stem for several reasons including the observation that the D form competitively inhibits the aminoacylation of the N form. The model which gave the closest fit between theory and experiment incorporates the feature that the DHU and the anticodon stem are replaced by a helix of five base pairs formed between residues in the T-Ψ-C loop and in the anticodon region. Conversion of the D to the N form would require the disruption of these five base pairs and could account for the high activation energy for this process. The validity of this model is strengthened by the fact that it is consistent with the oligonucleotide binding data reported by Uhlenbeck *et al.* (*Fed. Proc.*, **31**; 1972).

From a Correspondent

Nuclear and messenger RNA

THERE has long existed a prejudice that in higher animals, and in particular in birds and mammals, messenger RNA is first synthesised as a very much longer precursor RNA molecule, usually called heterogeneous nuclear RNA (HnRNA) because of its large and variable size. This is broken down in the nucleus to give mRNA (to be exported to the cytoplasm) and degraded fragments. Harris (*Biochem. J.*, **84**, 60; 1962) noted more than ten years ago that most of the RNA synthesised in the nucleus is broken down there and never exported to the cytoplasm. The nuclear RNA, however, is for the most part very large, and in sucrose gradients sediments at up to 70S (molecular weight approximately ten million); therefore, most of it might be degraded and yet the remainder yield molecules of the size of cytoplasmic messenger RNA (molecular weight about 250,000).

The original investigations of the relations between nuclear and cytoplasmic RNAs were carried out, in particular, by Darnell, Georgiev and Scherrer. Darnell has recently reviewed both the history and the evidence of the existence of precursor mRNA in the nucleus in a very lucid article (*Science*, **181**, 1215; 1973).

Direct evidence for the existence of mRNA in a large molecule in the cell nucleus can only come by a definitive identification of a specific messenger RNA, either by its translation into a protein or its sequence identification by DNA:DNA hybridisation or fingerprinting. The fact that a portion of the high molecular weight RNA of the nucleus contains poly (A) at its 3′ end certainly suggests a relation between it and cytoplasmic messenger RNA (all of which contain poly(A) sequences apart from histone mRNAs), but is not a proof positive. It is particularly important to show that RNA from the cell cytoplasm has not aggregated in a non-specific way with other nuclear RNA to give large mRNA-containing material which is not a primary transcript.

Three groups have now published data directly translating specific proteins from mRNA. Stevens and A. Williamson (*Nature new Biol.*, **245**, 101; 1973) have made use of the very tight binding found between heavy chain immunoglobulin mRNA and the complete myeloma protein (H_2L_2). In this way they purified the nuclear precursor RNA for the heavy chain mRNA and shown it to have a molecular weight of two million or greater. The nuclear pre-messenger causes immunoglobulin synthesis in *Xenopus* oocytes when injected.

In a similar series of experiments, R. Williamson and his colleagues (*Nature new Biol.*, **241**, 66; 1973) have microinjected HnRNA from mouse foetal liver cells into oocytes and obtained mouse globin synthesis. Since the RNA was purified using a zonal rotor, non-specific contamination is unlikely. Brain HnRNA prepared in the presence of reticulocyte polysomes synthesising mouse globin did not cause globin synthesis in oocytes.

Neither of these sets of experiments completely rules out the possibility of a specific interaction between a small nuclear mRNA and a larger nuclear RNA molecule which, for instance, transports it into the cytoplasm. In order to prove that such interactions are not causing the apparent large size of the nuclear RNA, it is necessary to show that these are still as large even when treated with solvents which break non-covalent interactions. Such hydrogen bond-breaking solvents include dimethylsulphoxide and formamide.

Ruiz-Carrillo and his colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3641; 1973) recently published a conclusive demonstration that large nuclear RNA from duck reticulocytes (unlike mammalian reticulocytes, those from birds have nuclei) contains globin mRNA sequences, even after centrifugation through a gradient containing 99% dimethylsulphoxide. Such a solvent would certainly denature all hydrogen-bonded aggregates, whether arising non-specifically during RNA isolation or during the synthesis of the RNA. Even nuclear RNA sequences larger than 45S code quite efficiently for duck globin in a cell-free protein synthesis system derived from mouse Krebs tissue culture cells. When rabbit globin mRNA, which sediments at 9S (molecular weight of 220,000), is added to the RNA before centrifugation, it does not contaminate the large nuclear RNA, as shown by the absence of rabbit globin synthesis.

As is well known to readers of these columns, an enzyme from RNA tumour viruses, reverse transcriptase, can make an exact complementary DNA copy (cDNA) from a messenger RNA template using an oligo(T) primer. Such a complementary DNA, which can be synthesised at a high level of radioactivity (up to approximately 80 million counts per min per microgram), can then in turn be used as a probe to search for messenger RNA or messenger gene sequences, even if these are present in very low amounts. The first attempts to utilise such techniques used not cDNA made with reverse transcriptase, but what might be called cRNA prepared with RNA polymerase (Melli and Pemberton, *Nature new Biol.*, **236**, 172; 1972). These results demonstrated the existence of globin mRNA sequences in duck nuclear RNA, but in this case disaggregating conditions were not used.

Scherrer and his colleagues (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 1122; 1973) used cDNA prepared from duck globin mRNA and annealed it to different size classes of large duck nuclear RNA. They showed that small amounts of globin mRNA sequences could be found even in RNA of molecular weight approximately ten million, although only perhaps 1% of the molecules of this size contained a globin mRNA sequence. The large nuclear RNA had to be denatured before the interaction could take place, indicating that it might be bound to other RNA sequences intramolecularly.

Recently two studies of the physical behaviour of giant nuclear RNA have appeared, one using material from sea urchins (Peltz, *Biochim. biophys. Acta*, **308**, 148; 1973) and the other from rat ascites cells (Holmes and Bonner, *Biochemistry*, **12**, 2330; 1973). In both cases very convincing evidence is presented that the RNA is still very large after denaturation, and is not an aggregate of messenger-sized molecules. Such evidence must encourage those who have argued for a long primary gene transcript, much longer than the ultimate cytoplasmic messenger.

Many questions are, however, still unanswered—Darnell outlines some in his paper in *Science*. I shall focus on just two. What is the significance of the large amount of secondary structure found in nuclear RNA? Might it represent a signal which determines the sites of cleavage before processing to the cytoplasm, perhaps ancillary to the poly(A) sequence and related to it? (It is known that short oligo(U) sequences do exist in large nuclear RNA, and might, of course, be signals which interact with the poly(A) at the 3′ end. It is surely interesting that the enzyme which processes RNA in *Escherichia coli* is a double-strand-specific RNase II (Dunn and Studier, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3296; 1973).)

The second question is the one which has always been intriguing—why is so much RNA synthesised anyway? It is known that *in vivo* as much as 10 or 15% of the DNA of the cell is transcribed, yet only a tiny fraction of this has a known function. Now it can be said with some certainty that each mRNA molecule contains, when made, a tail some ten or twenty times larger than itself, again of unknown usefulness. Moreover, only a proportion of the nuclear HnRNAs, even in a differentiated cell, contain poly(A). All of the recent attention has focussed on proving the existence of the messenger portion of the HnRNA. In future it may be the other sequences, among which controlling elements might be found, that will command more interest.

R.W.

Formation of the Himalayas

from our Geomagnetism Correspondent

IN their landmark paper on plate tectonic mountain building, Dewey and Bird (*J. geophys. Res.*, **75**, 2625; 1970) took the formation of the Himalayas to be a typical example of continent-continent collision, notwithstanding the fact that many workers have pointed to differences between the tectonic histories of the Himalayas and the Alps. But as Powell and Conaghan (*Earth Planet. Sci. Lett.*, **20**, 1; 1973) have now pointed out, although the Himalayas are obviously not cordilleran-type mountains, some of the criteria set up by Dewey and Bird and by Dewey (*Earth Planet. Sci. Lett.*, **6**, 189; 1969) to distinguish between collision and cordilleran belts are apparently not applicable in this case. For example, in cordilleran mountains gravity slides predate hard rock thrusting whereas in collision mountains the opposite occurs; the dominant metamorphic assemblages in collision mountains are in the blueschist facies in contrast to the paired metamorphic belts of cordilleran mountains; and whereas subduction continues during the formation of cordilleran mountains, it is terminated in collision mountain building by the buoyancy constraints on substantial underthrusting of continental crust. The strict application of these criteria would thus suggest that the Himalayas were not formed as a result of continent-continent collision—a conclusion which would clearly be wrong.

So how can this paradox be resolved? The answer, according to Powell and Conaghan, is that although the Himalayan mountain chain is basically of the collision type it was not formed as a direct result of the collision but as a result of uplift during underthrusting along a deep crustal fracture. The development of the Himalayas apparently took place in two stages. During the first of these the northward-drifting Indian subcontinent converged on and ultimately collided with a proto-Tibetan landmass. The second stage then began with the formation of a fundamental crustal fracture within the Indian block and continued with the underthrusting of the Indian subcontinent along this fracture.

In greater detail, the model proposed by Powell and Conaghan, and based on an examination of the structural and stratigraphic record, begins with the situation before 130 million years ago. At this stage the Indian block was drifting northwards towards the Asian continental block, the two land masses being separated by the shrinking Tethys Ocean. The stratigraphic record in the Spiti area of what is now northern India and southern Tibet indicates that open marine sedimentation was taking place on the continental terrace at the north of the Indian block during the Triassic and Jurassic, the deposits being predominantly carbonate and fine-grained terrigenous rocks. Fossils at the base of the Giumal Sandstone which overlies these deposits are no older than 130 m.y. and fossils in the higher Chikkim Series are no younger than 65 m.y., showing that the Indian and Asian blocks were still converging between these dates. The stratigraphic record in the vicinity of the present Indus suture indicates that throughout this period subduction was taking place at the southern edge of the Asian block—that is, subduction of the northward-drifting lithosphere carrying India.

The collision of India and Asia along what is now the Indus-Brahmaputra suture occurred before the middle Eocene (about 45 m.y.), but it seems unlikely that any mountains were thus produced. According to the Powell-Conaghan model, the first result of this original collision was a down-buckling at the margins of the Indian and Asian blocks as the subducting Indian plate, constrained by the buoyancy of its continental crust, became jammed. This led directly to the middle Eocene marine transgression of northern India, but by this time neither the central gneisses and schists of the High Himalaya nor the southward thrusts of the Lesser Himalaya had formed.

The period between the middle Eocene and the early Miocene (about 20 m.y. ago) was marked less by relative movement between India and Asia than by post-collision isostatic adjustment lead-

An Everest panorama (copyright: *The Times*).

ing to the Oligocene regression. It was during this period also that the gneisses and schists of the High Himalaya were produced, although the precise mechanism of formation has not yet been worked out. In any event, the narrow gneiss-schist zone formed some way to the south of the northern margin of the original Indian block, effectively splitting off the northernmost 100 km or so of the Indian crust. By the early Miocene the reduced frictional resistance in the gneiss-schist zone enabled the southern segment of the Indian lithospheric block to begin underthrusting; and by 5 million years ago it had underthrust both the original northern segment of the Indian crust and part of the Asian crust (not lithosphere). Where underthrusting had occurred there was, of course, a double layer of crust; and the resulting gradual isostatic uplift added to the upthrust of the Himalayas which had commenced as soon as the underthrusting began, as well as producing the Tibetan plateau. In fact, the Himalayas did not reach their present elevation until late Pliocene or Pleistocene, which fact led Gannser (*Geology of the Himalayas*, Wiley-Interscience, 1964) to speculate that prehistoric man may have witnessed their rise.

This is, of course, just an outline of the processes described in greater detail by Powell and Conaghan, who go on to give supporting evidence from seafloor spreading rates and from the sedimentation patterns in the deep sea fans associated with the Indus and Ganges/Brahmaputra river systems. But the central point is clear—the Himalayas were produced not directly by the continent-continent collision but indirectly by the subsequent underthrusting. As a result, since the early Miocene the active tectonic zone has been not the original site of continental collision marked by the Indus-Brahmaputra suture but the central crystalline axis of the gneiss-schist zone marking the High Himalaya (where current seismic activity is greatest). A more detailed consideration of the processes involved also explains why the Himalayan chain fails to conform with the three Dewey-Bird criteria for recognising single-stage collision-type mountain building.

Transitions in the sarcoplasmic reticulum

from a Correspondent

MEASUREMENT of the effects of temperature on transport processes in several microbial systems has led to correlations between mobility of lipids and transport activity. More detailed experiments with purified transport systems are beginning to appear. Even with these simplified systems the results are interpretable only in the most general terms, as shown by a thorough examination of the Ca^{2+} transporting vesicles from sarcoplasmic reticulum (Inesi, Millman and Eletr, *J. molec. Biol.*, **81**, 483; 1973). These vesicles contain few proteins and the major one, a Ca^{2+} activated ATPase which accounts for 70% of the membrane protein, is by itself able to transport Ca^{2+} across the membrane. The lipids are highly unsaturated and little cholesterol is present.

In earlier papers Inesi *et al.* reported changes in the slope of Arrhenius plots of the motion of several different nitroxide spin labels either linked to the $-$SH groups of the ATPase or incorporated into the lipid bilayer. The spin-labelled fatty acids in the lipid bilayer gave a change in slope at about 20°C which was not affected by prior heat denaturation of the protein. The maleimide spin labels coupled to the ATPase protein also showed a slightly increased slope below 20°C, and a more marked one above 40°C. The low temperature change seems to be a function mainly of the lipid phase since it can be correlated approximately with a change in rate of Ca^{2+} transport by a lipid soluble ionophore (Scarpa *et al.*, *J. gen. Physiol.*, **60**, 735; 1972). The nature of the change is obscure since the main order-disorder transition in systems of highly unsaturated lipids is below −20°C, but a parallel may be provided by changes in physical and transport properties of other unsaturated lipid systems in the neighbourhood of +20°C (for example, Esfahani *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **68**, 3180; 1971).

In their latest paper, Inesi *et al.* have correlated these two transitions with changes in ATPase activity and in the rate of Ca^{2+} transport. When the temperature was increased above 20°C, the heat of activation of both processes decreased from the very high value of 27 kcal mol^{-1} to 17 kcal mol^{-1}. It therefore seems that changes in the lipid environment are reflected in changes in activity of the protein. Above 40°C, net Ca^{2+} uptake ceased but ATPase activity continued unchanged; other evidence suggested that a change in the protein was involved. It is of interest that in the course of their recent determination of the diffusion constants of phospholipids in sarcoplasmic reticulum membranes, Scandella, Devaux and McConnell, *Proc. natn. Acad. Sci. U.S.A.* **69**,, 2056; 1972) found that this loss of Ca^{2+} transport at 40°C was prevented by 30% sucrose. This is but one of many examples of the sensitivity of this system to changes in the environment.

Although these transitions undoubtedly reflect interesting happenings within the sarcoplasmic reticulum, it will not be possible to interpret them in molecular terms until one has a better understanding of the structure of the ATPase protein and can incorporate it in a fully functional state into well sealed vesicles of purified lipids.

Quick measurement of carrier mobility

from our Solid State Physics Correspondent

AMONG experimental solid state physicists, the interpretation and measurement of carrier mobility in semiconductors or insulators has an aura of difficulty. Not only is the path of an electron, drifting in a field within a solid, influenced by many different forms of scattering (for example interaction with phonons, inpurities and vacancies in a variety of charge states) but the measurement is not simple. For the usual Hall method, precision-cut samples with at least four low resistance ohmic contacts are needed and the resulting 'Hall mobility' has to be corrected in order to find actual drift mobility. This characteristic of carriers must, however, still very frequently be determined during research on semiconductors and so much time and patience are expended by physicists in getting valid mobility data. That is why, when a 'quick' method for the measurement of mobility is announced, interest is naturally aroused.

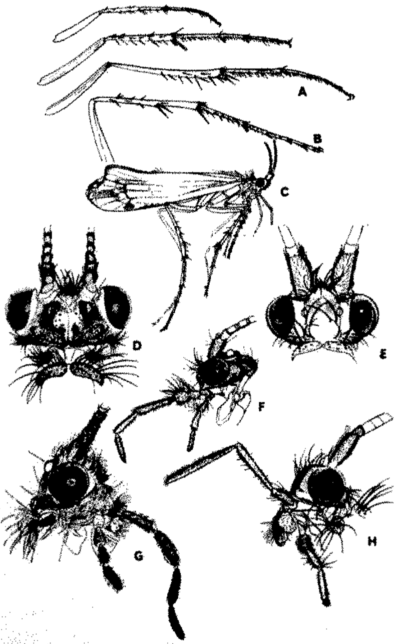
Howes (*Rev. Scient. Instrum.*, **44**, 1223; 1973) has described an evaluation of a 'direct-reading' method, using measurement of magneto-resistance by a nulling technique, involving two terminals rather than four. The latter feature brings advantages over and above the obvious one that preparation time is reduced. Many gallium arsenide transferred-electron devices, made for microwave generation studies, already have the right geometry of semiconductor chip and thus two correlated measurements can be performed on the same sample. This is particularly important when, as is often the case, different samples, even if cut from the same semiconductor ingot, may exhibit different impurity concentrations and carrier mobility values.

The new method relies on the fact that, for a sample of low aspect ratio (distance between terminals small compared with other dimensions), the applications of a magnetic field produces a reduced current flow. This results from the fact that, unlike the case of 'Hall bars' (which have a high aspect ratio) the transverse 'Hall Field' is not sufficient to overcome the Lorentz force which always reduces the near free path of carriers in the direction of the current flow. The decrease of this mean free path is reflected in the current passed for a given voltage and, in fact, a simple expression can be derived in which the absolute value of mobility is proportional to the square root of the sample resistance change (as a fraction of the original resistance and using unit magnetic field). It is thus a simple matter to balance a resistance bridge with the sample in one arm at zero magnetic field, then apply the magnetic field and use electronic means to rebalance the bridge. The electronics can then indicate the resistance change directly or, by using a circuit which performs a square-root operation, indicate directly the absolute value of 'magnetoresistance mobility'.

Howes has compared Hall mobilities with the magnetoresistance mobilities for several samples of germanium, silicon, gallium arsenide and indium phosphide and found that the ratio of the two values rarely varies more than 5% from unity. Thus, the method can at worst be described as a rough-and-ready Hall measurement and, at best, could make some tedious forms of Hall measurement unnecessary.

Key to caddis

LIMNOLOGISTS who have been brought up with the Scientific Publications of the Freshwater Biological Association will welcome the latest addition to the series—a key to the adults of the British Trichoptera by T. T. Macan (No. 28, £1.25). Caddis flies are not the easiest of freshwater animals to identify and this key, illustrated with drawings of specimens which have not been allowed to shrivel, should greatly simplify study of the order. In this figure, one of many drawn for the key by C. Joan Worthington, (*a*) legs of *Mesophylax impunctatus*; (*b*) mid-leg of *Agrypnia pagetana*; (*c*) *Glyphotaelius pellucidus*, preserved specimen; (*d-h*) heads from above and from side; (*d*) *Phryganea varia*; (*e*) *Limnephilus rhombicus*; (*f*) *Rhyacophila dorsalis*; (*g*) *Phryganea varia*; (*h*) *L. rhombicus*.



Integration of proviruses

from a Correspondent

IT is now 10 years since Temin postulated that RNA tumour viruses replicate by way of a DNA intermediate, the provirus. This hypothesis, for many years regarded as heresy, gained general acceptance in 1970 with the discovery of an RNA-dependent DNA polymerase (reverse transcriptase) in RNA tumour virus particles. Last year, Linial and Mason (*Virology*, **53,** 258; 1973) reported that the reverse transcriptase does indeed play an essential part in the function of these viruses because mutants of Rous sarcoma virus, which are temperature sensitive for the enzymatic activity, cannot replicate in nor transform cells infected at the non-permissive temperature.

It has proved more difficult, however, to demonstrate the formation of proviruses inside infected cells. Until recently, attempts to find new proviruses by molecular hybridisation have been thwarted by the presence of endogenous viral genetic information in the natural host cells. Schincariol and Joklik (*Virology*, **56,** 532; 1973) have now demonstrated the formation in infected cells of additional sequences of DNA homologous to the RNA of Rous sarcoma virus (RSV). Schincariol and Joklik used a sensitive single-stranded DNA probe synthesised from RSV RNA by reverse transcriptase in the presence of actinomycin D. The DNA represents more than 90% of the RNA genome. Reassociation kinetics of the complementary DNA in the presence of excess DNA prepared from infected and uninfected chick cells indicate that two copies of RSV provirus are made in addition to the one viral genome equivalent endogenous in chick cells. By hybridising excess RNA prepared from cells at different times after infection to the single-stranded DNA probe, Schincariol and Joklik also examined the initiation of transcription of the proviruses and found that 100 copies were transcribed within 24 h of infection.

The complicating factor of endogenous viral genetic information may be avoided by infecting unnatural host cells; for instance, mammalian cells do not have DNA homologous to avian tumour virus RNA. Thus Varmus, Vogt and Bishop (*J. molec. Biol.*, **74,** 613; 1973) found two to four avian proviruses in mammalian cells transformed by RSV but none in uninfected cells. Varmus, Vogt and Bishop (*Proc. natn. Acad. Sci. U.S.A.*, **70,** 3067; 1973) now report that the viral DNA becomes covalently linked to host DNA following transformation of mouse, rat, or duck cells with RSV. Varmus *et al.* make use of Britten's observations that most high molecular weight DNA extracted from higher organisms contains reiterated sequences. When unsheared DNA is incubated to low C_0t values at which repeated, but not unique, sequences reassociate, 'networks' of DNA are formed which can be separated from the remainder of the DNA by sedimentation. Much of the DNA homologous to viral RNA in transformed cells was found in the networks, thus demonstrating its integration into the host genome.

Varmus *et al.* then examined the formation and integration of viral DNA in freshly infected cells. Duck cells are permissive for replication of certain strains of RSV and were found to contain no endogenous sequences homologous to the RNA of RSV. Virus-specific DNA was detected within 3 h of infection and using the 'network' assay, integration of viral DNA was detected from 6 h onwards. Mouse 3T3 cells are not permissive for RSV replication and have a very low efficiency of transformation by RSV, relative to chick or duck cells. Readily detectable levels of RSV DNA, however, were found in 3T3 cells within 12 h of infection, though the RSV DNA did not become integrated until several hours later. These observations suggest that the RSV particles enter and form proviruses in 3T3 cells at an efficiency comparable to that in permissive cells, although transformation is less than one thousand times as efficient. It will be interesting to see whether viral RNA is transcribed off the proviruses in these 'silent' infections.

Integration of the DNA provirus provides an explanation how RNA tumour viruses, like DNA tumour viruses, can become preserved in infected cells and cause stable transformation. Does this mean that all integrating viruses could be tumour viruses? One exception is Visna virus, which causes a 'slow' demyelinating disease in sheep. It has not been associated with any neoplastic disease although Takemoto has observed cell transformation in culture by Vinsa. Visna is an RNA virus that resembles RNA tumour viruses in structure and carries a reverse transcriptase. Haase and Varmus (*Nature new Biol.*, **245,** 237; 1973) have investigated the replication of Visna virus during lytic infection off sheep choroid plexus cells.

Using the 'network' hybridisation method, they show that it forms a DNA provirus which becomes integrated into the host genome.

Vanishing interstitial in semiconductors

from a Correspondent

Recent work by a group of American workers (Weigel *et al., Phys Rev. B*, **8**, 2906; 1973) helps to clear up one of the current problems in solid state physics—the mystery of the vanishing interstitial in semiconductors.

When a crystal is irradiated with high energy electrons or other particles, atoms are knocked off their lattice sites. The simplest defects thereby created are the Frenkel pair—the vacant lattice site (the vacancy) and the ejected atom (the interstitial, so called because it occupies one of the interstices of the lattice rather than a normal substitutional site). Of the elemental semiconductors (diamond, germanium and silicon) the latter has received most attention experimentally and vacancies have been detected, both alone and in combination with other defects. The problem is that nobody has yet observed the interstitial, in spite of many attempts to do so. It seems that this defect is very mobile and can diffuse over long distances until it recombines with a vacancy or is trapped by a dislocation or reaches the surface of the crystal, thereby disappearing. But as everybody is aware these days, the ability to travel a long way depends on the availability of a supply of energy. In the normal atomic transport mechanism the energy is thermal; the transistors are made by diffusing in impurities at temperatures in the region of 1,000° C. But the silicon interstitial seems to be mobile at 4 K, making it unlikely to be a thermal process.

The calculations of Weigel *et al.* are for carbon (diamond) because the approximation used, Extended Hückel Theory, has been very successfully applied to organic molecules; but the results are expected to apply to silicon and germanium as well. The solution of the mystery has two aspects. First, one would expect that an interstitial atom would occupy the largest interstice available. In the silicon lattice this is the tetrahedral site, equidistant from four lattice atoms. But the calculations show that this is not the case. The interstitial combines with a lattice atom, pushing it slightly out of position. Neither of the atoms occupies the lattice site but are both symmetrically displaced from it along the ⟨100⟩ crystal direction (the 'split-⟨100⟩' interstitial), rather like two people trying to sit on one chair. This situation is of lower energy than one lattice atom plus an interstitial and hence is more stable. It also explains why previous calculations, which assumed a tetrahedral site, were not very successful.

The second aspect of the solution is that there is not one stable situation, but two, the other being the 'bond-centred' configuration, where the interstitial sits midway between two nearest neighbour lattice atoms which are symmetrically displaced from their sites, like three people sitting on a two-seater settee. Furthermore, which of these two 'interstitialcy' configurations has the lower energy depends on the charge state and this can change during irradiation due to the tremendous amount of ionisation created. Consequently the interstitial would switch from one configuration to the other and hence migrate through the lattice whatever the temperature. This mechanism, 'ionisation-enhanced diffusion', was suggested earlier by Bourgoin and Corbett (*Phys. Lett.*, **38**A, 135; 1972).

The authors are properly cautious about their results. For example, Extended Hückel Theory is of necessity a rather simple approximation because of the complicated nature of the calculations. And strictly speaking the extension to different charge states is not valid. It seems likely, however, that a more rigorous calculation would reach the same conclusions.

Nature of scrapie agent

from a Correspondent

Work on the agent of scrapie, one of the spongioform encephalopathies, continues to be both intriguing and frustrating. At a meeting of the British Photobiology Society on January 16 at Imperial College, London the latest attempts to characterise the active macromolecules of this agent were discussed. Two introductory lectures by I. A. Pattison and D. A. Haig (both of the ARC Institute for Research on Animal Diseases, Compton) outlined the pathogenesis of scrapie and in particular how it differs from conventional virus diseases. Haig and his colleagues have found that the scrapie agent (SA) multiplies in cultured mouse brain cells but with one remarkable difference from normal viral proliferation—there is a strict correlation between the number of cells and SA activity. As the cells divide, the infectivity increases on a one for one basis. Approximately 100 cells are required to cause infection in a test animal.

G. D. Hunter, also from the Institute for Research on Animal Diseases, favours a structure for the SA which involves a small piece of RNA closely associated with plasma membrane. His conviction is based on his own work, which has shown conclusively that the plasma membranes are the sites of the SA, and on work by T. O. Diener in the United States on the very small host-dependent potato RNA viroids which exhibit some of the physico-chemical properties of the SA. Some caution must be observed, however, when comparing the properties of the RNA potato viroids, which are extracted in a relatively pure form by classical nucleic acid techniques, with those of the SA which has proved difficult to isolate by such methods.

T. Alper (MRC Experimental Radiopathology Unit, Hammersmith Hospital) who, in collaboration with Haig, was responsible for early work in determining the size of the infective particle by the use of ionising radiation, reported that like some biologically important membrane systems the sensitivity of the SA to ionising radiation is much enhanced by the presence of oxygen when irradiated in aqueous suspension, whereas biologically active nucleic acids and proteins are typically no more sensitive.

Finally, R. Latarjet (Fondation Curie, Paris) suggested four possible structures for the SA which could explain why it is highly resistant to ultraviolent light. These were: a very small piece of nucleic acid: a larger piece of nucleic acid which suffers ultraviolet damage but is repaired in the host with high efficiency; nucleic acid protected by other constituents of a complex; and, lastly, a non-nucleic acid agent. Results of Latarjet's work in collaboration with Alper, Haig and Clarke, who attempted to characterise the SA by studying its response to various wavelengths of ultraviolet light, suggest that the SA may be non-nucleic acid in structure. Latarjet, however, could not at present rule out a combination of nucleic acid and glycoprotein where energy absorbed in the glycoprotein at wavelengths not characteristic for nucleic acids is transferred to the nucleic acid moiety.

Absorption of toxins by leaf surfaces

from our Plant Ecology Correspondent

It is well known that vegetation acts as a filter for particulate and aerosol material carried in the atmosphere. The effects of both impaction upon solid surfaces and settlement in situations of low wind speeds lead to the accumulation of material on the surfaces of plant leaves and stems. Suspended matter in the atmosphere may originate from the oceans, from wind eroded soils, or from industrial activities; some matter can be regarded as beneficial in that it is composed of substances of nutritional value to plants, whereas other materials are undoubtedly toxic. The arrival of such airborne matter in aneco system represents an input of nutrient or pollutant and its presence raises several questions of ecological interest. Is the nutrient or toxin input of significant importance in relation to the quantity and rate of movement of that substance in the system already? Is the vegetation which acts as a filter able to absorb the material through the leaves? Is the material washed from the canopy to the soil surface and, if so, is it taken up by plant roots? Of what importance is the material (particularly if it is a pollutant) to consumer organisms especially at the higher levels of food chains?

Goodman and Roberts (*Nature*, **231**, 287; 1971) found that considerable quantities of toxic metals can arrive at a vegetation surface as dust. Little (*Environ. Pollut.*, **5**, 173; 1973) has now raised further questions regarding the behaviour of metal contaminants within ecosystems. He attempted to ascertain whether zinc, lead and cadmium are present chiefly on the surfaces of leaves and can be removed by washing, or whether they are absorbed into leaf tissue. He also examined three different tree species in the Bristol area—*Ulmus glabra* (elm) *Quercus robur* (oak) and *Salix alba* (willow)—to find out whether they respond differently with respect to the three metals.

Little's report provides some useful information on the behaviour of toxins on leaf surfaces. Washing elm leaves in distilled water removed 67% of their total zinc burden, 87% of their lead and 62% of their cadmium. It seems therefore that the bulk of these metals is present as a deposit on the leaf surfaces and can be carried to the soil by rainwashing. Most of the remainder could only be removed by washing with dilute nitric acid, which implies that this fraction is bound more securely to the leaf surface, possibly by exchange phenomena. Boiling in deionised water for 1½ h removed the portions of the metals present in the cuticle and within cells; they comprised 7% of the total zinc, 2% lead and 4% of the cadmium in the elm leaves. This tendency for a greater degree of incorporation of zinc and cadmium into the leaf structure reflects the higher solubilities of these metals. The dust input of all these metals must therefore be regarded as high when compared with quantities already present in the vegetation.

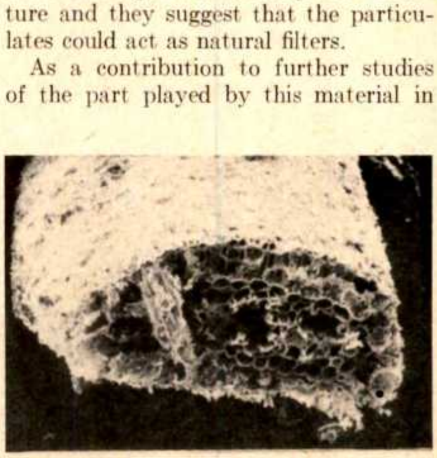
Other trees behaved rather differently with respect to the three metals; for example, hawthorn and willow retained larger proportions of zinc and lead after washing than did oak, which may be explained by the thicker, waxy cuticle of oak. Skarr *et al.* (*Nature*, **241**, 215; 1973) found that some bryophytes (which lack a waxy cuticle) absorb lead and store it in their nuclear membranes. Unfortunately, nothing is known of the uptake of these metals from the soil so that it is impossible to determine the relative importance of foliar absorption. Most of the matter washed from leaf surfaces accumulates in the superficial soil layers, as a result of which shallow rooting herbaceous species may be affected by soil toxicity more than deeper rooting trees.

A further study of dust accumulation, this time in a wetland ecosystem, is described by McLay (*Environ. Pollut.*, **5**, 173; 1973). In an area remote from industrial pollution in California, he examined a small alkaline lake where emergent *Scirpus californicus* grew around the edge and where *Lemna perpusilla* was abundant on the surface. Growth of *Lemna* was found to be significantly greater in the laboratory if washings from *Scirpus* stems were added to the lake water in which they were grown. McLay fails to demonstrate, however, that the stimulation is a result of additional nutrients from dust. It could quite easily be explained in terms of leaf exudates from *Scirpus*, either of an inorganic nature or as organic, growth promoting substances.

No smoke without fire

AN unusual line of research but one that may have environmental significance is the ecology of smoke particulates and charcoal residues from forest and grassland fires. E. V. and B. B. Komarek, who work at the Tall Timbers Research Station, Tallahassee, and T. C. Carlysle of the Gainsville Laboratory of the US Department of Agriculture, believe that airbourne particles from such fires act as environmental cleansing agents. They have found from electron micrography that the plant material which remains after burning has a varied porous structure and they suggest that the particulates could act as natural filters.

As a contribution to further studies of the part played by this material in the atmospheric ecosystem, Komarek *et al.* have prepared an atlas of particulates collected from controlled fires (*Misc. Publ. Tall Timbers Research Station*, No. 3; 1973). Although the airbourne material and the residues that remain on the ground vary from carbonized material to minerals, white ash, tars and gases, some of the original plant genera can be identified. Species of pine and hardwood trees, grasses and cultivated plants, for example, are represented in the atlas of 136 microphotographs. The figure here shows a cross section of a charred needle of spruce pine (*Pinus glabra*).



Breaking down bacterial messengers

from our
Molecular Genetics Correspondent

ALTHOUGH in most areas of nucleic acid synthesis studies of bacteria have lead the way and research with eukaryotic systems is only now following, the processing of RNA is an exception; for the maturation of ribosomal RNA by cleavage from a large precursor is well established in eukaryotes and it is evident also that messenger RNA is initially transcribed in the nucleus in a form much larger than its eventual cytoplasmic size. Two studies reported in the first part of the December *Proceedings of the National Academy of Sciences* now provide a welcome balance by throwing some light on an enzymatic activity which may be involved in processing RNAs in bacteria.

Using the DNA of phage T7 as a template, Dunn and Studier and others also have shown previously that the messenger RNAs synthesised *in vitro* are derived by transcription from the beginning of the DNA to a point about 20% along it, whereas the mRNA found *in vivo* consists of five smaller species which seem to be derived by cleavage of a precursor corresponding to the *in vitro* messenger. The enzymatic activity which breaks the precursor down seemed to be very similar to ribonuclease III, one of the known ribonucleases of *Escherichia coli*. Taking advantage of a mutant strain of *E. coli* deficient in this enzyme, Dunn and Studier now report (*ibid.*, **70**, 3296; 1973) that this enzyme is implicated in both T7 mRNA cleavage and in rRNA cleavage.

In spite of the absence of a paragraph apparently omitted from the beginning of the "Results" section (this issue of the *Proceedings* appears with an apology from the editors for its delayed publication and lack of consistency in conventions followed in different papers), it seems that Dunn and Studier have examined the T7 RNAs produced in normal and RNase III deficient strains of *E. coli* by gel electrophoresis. They find that the ribonuclease deficient strain the usual pattern of small RNAs is replaced by a large RNA identical with that usually transcribed *in vitro*. Extracts from *E. coli* strains containing ribonuclease III can cleave this large RNA to generate the messengers usually found *in vivo*.

Another maturation event, studied both by Dunn and Studier and also by Nikolaev, Silengo and Schlessinger (*ibid.*, **70,** 3361; 1973), seem to take place in ribosomal RNA synthesis. In the RNase III deficient strain, synthesis of irbosomal RNA is slowed and the usual 16S and 23S sedimenting molecules are replaced by a precursor of 30S or 1.8 – 2.3 × 10^6 daltons (according to the different estimates), large enough to contain both sequences. Hybridisation experiments confirmed that the giant molecule competes with both the mature species. Cleavage *in vitro* with an extract of strains possessing RNase III showed that this activity can generate two molecules of about the size of 16S and 23S rRNAs.

The production of ribosomal RNA in *E. coli* is thus very similar to the pathway previously characterised in eukaryotic cells, although the eukaryotic precursor is some twice the combined size of the mature molecules, so that a great amount of additional material is degraded. It is not clear just how much larger the bacterial precursor is compared with the combined mature molecules. One puzzling feature of these results is that precursors only slightly larger than the 16S and 23S rRNAs have previously been described, a point mentioned in neither of the present articles. One model is to suppose that an initial cleavage generates the precursors found, which are then further degraded and modified to yield mature ribosomal RNAs.

That the cleavage of precursors to yield mature RNAs is found also with a phage messenger is, as well as its importance in excluding other mechanisms previously proposed for phage mRNA synthesis, important for its implication that this might occur with bacterial messengers. Any molecule of RNA possessing the specific sequence recognised by ribonuclease III will presumably be cleaved by the enzyme; future research will no doubt elucidate the recognition sequence and investigate its possible presence in bacterial messengers.

Another striking claim concerning the metabolism of RNA is made by Downey *et al.* (*ibid.*, **70,** 3400; 1973), who report that a cytoplasmic RNA dependent RNA polymerase—in other words an RNA replicases—can be found in lysates of rabbit reticulocytes. The enzyme seems to utilise RNAs as templates *in vitro* to direct incorporation of labelled triphosphates into an acid precipitable product. The enzyme proved inactive when provided with DNA as primer.

The presence of an RNA replicase in the cytoplasm would add a new level of control to expression and could, for example, account for production of haemoglobin after synthesis of mRNA has ceased (instead of postulating that the haemoglobin mRNA is stable). But before this conclusion can be accepted it will be necessary to categorise rigorously the product of the reaction by qualitative means such as hybridisation assays instead of the only preliminary quantitative assay of total nucleotide incorporation. And of course, it is necessary to bear in mind frequent demonstrations of recent years that activities *in vitro* make few implications about enzyme roles *in vivo*.

Function of chloroplast DNA and its replication

from a Correspondent

ALTHOUGH the presence of DNA in chloroplasts has been recognised for more than a decade, surprisingly little is known about its function. Measurements of the molecular weight of the chloroplast genome generally give values of approximately 1–2 × 10^8 daltons. This is sufficient to code for several hundred proteins, yet isolated chloroplasts have only been shown to synthesise the larger subunit of the fraction 1 protein and two membrane-associated proteins (Blair and Ellis, *Biochem. J.*, **127,** 42P; 1972; Ellis and Forrester *ibid.*, **130,** 28P; 1972).

It seems probable that many chloroplast enzymes are coded for by nuclear genes. The genetics of the fraction 1 protein, which catalyses the carboxylation of ribulose diphosphate, are particularly interesting. The gene for the larger subunit of the protein shows cytoplasmic inheritance and is presumably located in the chloroplast whereas the gene controlling the smaller subunit shows Mendelian inheritance and is therefore in the nucleus (Kawashima and Wildman, *Biochim. biophys. Acta*, **262,** 42; 1972; Chan and Wildman, *ibid.*, **277,** 677; 1972). Another part of the chloroplast DNA codes for the 1.1 × 10^6 and 0.5^6 × 10^6 dalton RNA components of the chloreplast ribosomes (Scott and Smillie, *Biochem. biophys. Res. Commun.*, **28,** 598; 1967) and these RNAs have been shown to be synthesised inside chloroplasts (Hartley and Ellis, *Biochem. J.*, **134,** 249; 1973).

On the other hand DNA-RNA hybridisation studies with higher plant systems have previously suggested that a large number of genes for chloroplast ribosomal RNA are also present in the nucleus although there are alternative explanations of these results (Tewari and Wildman, *Proc. natn. Acad. Sci. U.S.A.*, **59,** 569; 1968; Ingle *et al. Symp. Soc. exp. Biol.*, **24,** 303; 1970). Experiments by Scott now suggest that at least in *Euglena* chloroplast ribosomal RNA may be coded for only by chloroplast DNA (*J. molec. Biol.*, **81,** 327; 1973).

Of the total DNA in light-grown *E. gracilis* cells, between 5 and 6% is present in chloroplasts. There are about ten chloroplasts in each cell and each organelle contains 40–80 copies of the chloroplast DNA which has a molecular weight between 1.8 × 10^8 and 0.7 × 10^8 daltons. DNA isolated from the chloroplasts has an average buoyant density in caesium chloride of 1.686 g cm^{-3}, which distinguishes it from the nuclear DNA with an average density of 1.707 g cm^{-3}. When RNA from chloroplast ribosomes is hybridised to denatured chloroplast DNA immobilised on nitrocellulose filters, the saturation values give an estimate of 6% of the DNA coding for chloroplast rRNA. These genes are clustered on the DNA and have a buoyant density of 1.696 g cm^{-3}. There are separate cistrons for the larger and smaller RNAs with between three and six copies of each cistron per chloroplast genome depending on which value for the size of the genome is correct. This works out at 2,400 genes for chloroplast rRNA per cell. By contrast there are 1,000 genes, buoyant density 1.716 g cm^{-3}, coding for the RNA of the cytoplasmic ribosomes. Such a multiplicity of rRNA genes is commonly found in eukaryotes and is consistant with a high metabolic demand for ribosome synthesis.

Euglena cells grown heterotrophically in the dark do not develop chloroplasts. When DNA from dark-grown cells is challenged with chloroplast rRNA, Scott found that although there is some hybridisation this represents a non-specific reaction. Heating the 'hybrids' dissociates the DNA-RNA duplexes and the melting profile thus obtained affords a measure of the degree of base paring involved. The results clearly show that the duplexes have a much lower thermal stability than authentic rRNA–rDNA hybrids.

The conclusion is that DNA from dark-grown cells (largely nuclear DNA) does not contain a large number of genes for chloroplast rRNA. Presumably there must be at least one copy of the chloroplast DNA present in dark-grown cells but even several copies could have remained undetected in these experiments. For this reason the possibility that there are a very small number of chloroplast rRNA genes in the nucleus cannot yet be ruled out.

Cosmic Far Ultraviolet Background

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Observations of a diffuse ultraviolet background at high galactic latitude might reveal the existence of an intergalactic medium. Although existing results provide only weak constraints, current techniques are sufficient to set interesting limits on its density and temperature.

INTEREST in the diffuse far ultraviolet background stems mainly from its relevance to the widely held view that intergalactic space may be filled with a dilute gas containing the majority of the mass of the universe. Observations which might reveal the existence of the intergalactic medium (IGM) have been attempted at radio, optical, far ultraviolet and X-ray wavelengths (see ref. 1 for a recent review). If the IGM has a density comparable to the critical density required to close the universe, then 21 cm radio observations and optical observations of QSO spectra imply the gas is highly ionised and therefore $T \gtrsim 10^4$ K. On the other hand observations of the diffuse X-ray background show that the temperature of such a gas is probably less than about 3×10^8 K. In the lower part of this acceptable range of temperatures, the IGM would radiate primarily in the far ultraviolet. Alternatively, the high degree of ionisation of the gas may be maintained by a large ultraviolet flux from some as yet unidentified source. Thus, the far ultraviolet region of the spectrum deserves considerable attention in the effort to determine the density and temperature of the IGM.

Since the first report[2] of an observation of the diffuse far ultraviolet background, there have been several measurements attempted, both longward and shortward of Lyman α. As the reported intensities range over nearly three orders of magnitude, a critical evaluation is required before the observations may be interpreted in terms of limits on the physical state of the IGM. Here we discuss the expected intensities of various possible components of the far ultraviolet background and review the relevant observations.

We conclude that existing results do not place interesting constraints on the density of the IGM. Current techniques and instrumentation for far ultraviolet astronomy are, however, sufficient to achieve vastly improved limits, but new observations are required to determine whether the IGM can be detected in the far ultraviolet or whether the extragalactic component of the background is masked by radiation with a more local origin.

There are several contributors to the far ultraviolet background radiation in addition to the possibility of emission from the IGM. The extragalactic background will include a contribution from galaxies and QSOs. A galactic background will be present which will consist of direct stellar radiation and a truly diffuse component resulting from starlight scattered by interstellar dust. Locally there will be contributions from the zodiacal light as well as airglow emissions from the Earth's geocorona. In what follows, we discuss briefly what is known, either theoretically or observationally, about each of the components of the far ultraviolet background.

Intergalactic medium

Attempts[3] to observe Lyman α absorption in the optical spectra of large redshift QSOs have led to the conclusion that the IGM is either remarkably empty, or it was highly ionised at $z \geq 2$. Such a high degree of ionisation implies temperatures greater than about 10^4 K. Kurt and Sunyaev[2] first discussed Lyman α radiation from recombinations in the IGM. Emission in this line is maximised at $T \approx 2\times10^4$ K, where an intensity of 4×10^{-10} erg (cm^2 s sr Å)$^{-1}$ just longward of Lyman α is produced in a uniform universe having the critical density and with the Hubble constant $H_0 = 50$ km s^{-1} Mpc^{-1}. The best current estimate[4] of the deceleration parameter in a Friedmann model of the universe is $q_0 = 1 \pm 1$ and this yields an intensity of 1.6×10^{-9} erg (cm^2 s sr Å)$^{-1}$.

Silk and Tarter[5] have considered a clumpy IGM, consisting of clouds of ionised gas which are distributed similarly to galaxies. In such a model, it is possible to gravitationally bind groups and small clusters of galaxies, as well as the universe, without violating limits on the ultraviolet and soft X-ray backgrounds. Since the clumpiness increases the emissivity of the IGM per unit mass, a substantial flux of Lyman α recombination radiation may be produced even at $T \gg 2\times10^4$ K.

The cosmic Lyman α flux is also relevant to the arguments which lead to the conclusion that a dense, neutral IGM cannot exist. Although the QSO observations already mentioned provide extremely strong limits on neutral hydrogen (n_H $(z=2) < 1.5\times10^{-11}$ cm^{-3}), this result depends on the assumption that QSO redshifts are cosmological. Even with this assumption, these data provide information only about a distant epoch. Since the cosmological distance assumption is questioned by some astronomers and since the ionisation of the IGM at the present epoch may differ from that at $z \simeq 2$, the limits on neutral hydrogen obtained through 21 cm observations of low redshift objects are still of interest. These observations[1] give $n_H/T_E < 1.8\times10^{-8}$ cm^{-3} K^{-1}, where T_E is the excitation temperature of the 21 cm line. The principal unknown contribution to T_E comes from recombination Lyman α in the IGM. Thus an upper limit on the Lyman α flux is required to set a limit on T_E and thus on n_H. An indirect limit of $T_E < 12$ K has been set by Field[1]. A direct observational confirmation of this requires the establishment of an upper limit of 4×10^{-10} erg (cm^2 s sr Å)$^{-1}$ for the cosmic Lyman α flux.

In addition to ionised hydrogen, the IGM is generally believed to contain about 10% helium which was produced in the primeval fireball. At a temperature of about 10^5 K the IGM will thus radiate strongly in the HeII λ 304 Å recombination line. Radiation of this wavelength, originating at epochs with $z \approx 2.5$ to 3.0, will be redshifted into the observable spectral region around 1,100 Å. Kurt and Sunyaev[2,6] first pointed out that because the density in an expanding universe varies as $(1+z)^3$ and the intensity of a recombination line varies as the square of the density, measurement of the redshifted helium radiation provides an even more sensitive probe of the IGM than that provided by the hydrogen radiation. Thus observations shortward as well as longward of Lyman α may yield information on the thermal history of the IGM back to epochs where the most distant QSOs have been observed.

Galaxies and QSOs

The possibility that the IGM is photoionised by far UV radiation from QSOs and galaxies has been considered by several authors[7-11]. Rees and Setti[9] estimate that QSOs alone would produce a Lyman limit intensity of about 4×10^{-11} to 15×10^{-11} erg (cm^2 s sr Å)$^{-1}$. They also suggest a similar contribution could be made by an extrapolation of the diffuse X-ray background spectrum to ultraviolet wavelengths. Such a flux would be sufficient to maintain a high degree of ionisation, consistent with the Gunn–Peterson result, in an IGM whose density is $n{\sim}10^{-7}$ cm^{-3}. Rees and Setti conclude that an IGM with the critical density could be sufficiently photoionised by a far ultraviolet background whose intensity is $\sim2\times10^{-9}$ erg (cm^2 s sr Å)$^{-1}$. The existence of such a universal ultraviolet flux might have profound implications beyond its effect on the IGM.

A suggestion that normal galaxies are strong emitters of far ultraviolet radiation has recently been made by Hills[12]. According to this work, ultraviolet dwarfs with temperatures of about 2×10^5 K emit enough ultraviolet photons to maintain a high degree of ionisation in an IGM having the critical density. This radiation, however, is strongly concentrated beyond the Lyman limit, and the intensity in the observable region at approximately 1,300 Å would be only about $\sim5\times10^{-12}$ erg (cm^2 s sr Å)$^{-1}$. On the other hand, if such hot stars were numerous in young galaxies at large z, their contribution to the intensity at approximately 1,300 Å might be substantially larger.

Although the intensity of the cosmic far ultraviolet background is not predictable, it is clear that a sensitivity of $\lesssim10^{-9}$ erg (cm^2 s sr Å)$^{-1}$ should allow some interesting conclusions to be drawn. An extragalactic background of this intensity, however, might be overwhelmed by galactic and even more local sources of ultraviolet radiation, thereby forever precluding investigation of the IGM at ultraviolet wavelengths. We turn now to a consideration of these other components of the ultraviolet background.

Galactic starlight

It is clear that observations made with detectors with large fields of view will include a spurious flux component arising from point sources whose space density is sufficiently large. The mean intensity of direct starlight in the solar neighbourhood at approximately 1,400 Å is about 1×10^{-7} erg (cm^2 s sr Å)$^{-1}$ according to calculations by Habing[13]. Other calculations[14] suggest a variation of a factor of about ten in intensity of direct starlight from galactic plane to pole. Thus, observations made with large field detectors may be expected to yield I(1,400 Å)$\gtrsim10^{-7}$ erg (cm^2 s sr Å)$^{-1}$ to within a factor of ten. This is consistent with the observations discussed below.

To observe the cosmic background, this pseudo background must be substantially reduced by using a small field of view. As the field of view is made smaller, sources of progressively higher space density are eliminated. This has a particularly important effect on far ultraviolet observations, as the relatively low density objects which may be completely eliminated with a small field of view are just those hot stars which contribute most to the ultraviolet flux measured with a large field detector. Of course, as the field of view is reduced, so is the sensitivity of the detector to diffuse radiation. There must therefore be an optimum field of view which is just small enough to eliminate a substantial contribution from stars.

For a field of view of 0.1 square degrees, only stars of spectral type F and later are numerous enough to contribute to the diffuse flux at high galactic latitude, but such stars are intrinsically weak in the far ultraviolet. If a telescope is oriented to avoid stars brighter than $V=10$, we expect the stellar contribution to the background will be less than approximately 10^{-11} erg (cm^2 s sr Å)$^{-1}$, substantially below the predicted extragalactic flux. Similar conclusions have been reached by Kurt and Sunyaev[6] and by Smirnov[15]. With this field of view the ultraviolet dwarfs discussed above will not be sufficiently numerous to produce a significant diffuse component either.

Diffuse galactic light

Extinction of far ultraviolet radiation in the galaxy is due to interstellar grains. Some models of these grains, such as dielectric particles and Platt particles, predict high albedos. If the extinction is mainly due to scattering, a diffuse galactic light will exist, whose origin is in starlight. If the albedo is very high, the energy density of this diffuse light may be comparable to that of the direct starlight. The intensity of diffuse light will vary with direction in the Galaxy in a way which depends on the distribution of hot stars, the distribution of the dust, the albedo, and the phase function for the scattering. For a simple model of the galaxy in which the stars and dust are similarly distributed in plane parallel layers, the variation of intensity of diffuse light with galactic latitude has been calculated by Van de Hulst and de Jong[14]. Taking the grain parameters predicted by Gilra's models[16] and a galactic half optical thickness of 0.2, we find from Van de Hulst and de Jong's work that the diffuse light at the pole is about 0.5% of the total intensity (including starlight) near the galactic plane. This intensity[17] is about 5×10^{-7} erg (cm^2 s sr Å)$^{-1}$ at 1,400 Å. Thus, the diffuse galactic light predicted at the pole in this model is 2.5×10^{-9} erg (cm^2 s sr Å)$^{-1}$, which is somewhat larger than the expected Lyman α flux from the IGM.

If the plane-parallel model were applicable, the diffuse galactic light would make a measurement of the cosmic far ultraviolet background difficult. It has been shown, however, that this model for the distribution of interstellar dust is inadequate. A wide variety of studies[18] suggest that the interstellar reddening $E(B-V)=0.00$ for galactic latitudes greater than $b=50°$. This result may easily be reconciled with the usual cosecant b dependence of the plane-parallel galaxy models when it is realised that the dust is concentrated in clouds. At low latitudes, where a line of sight intersects many clouds, the uniform distribution assumption is not inappropriate but at high latitudes most lines of sight simply do not intersect a dust cloud. Thus, for most directions above $b=50°$ no diffuse galactic light is expected and therefore the cosmic UV background will not be masked.

Zodiacal light

The zodiacal light results from solar radiation scattered by interplanetary dust and is one of the principal contributors to the sky brightness at visual wavelengths. Because of the sharp decrease of the solar flux toward short wavelengths, however, it may be expected that the zodiacal light will not contribute much to the far ultraviolet background. Thus, Kurt and Sunyaev[6] concluded that the intensity of the zodiacal light at 1,300 Å is less than 2×12^{-12} erg (cm^2 s sr Å)$^{-1}$. On the other hand, Lillie[19] presented preliminary evidence that the zodiacal light spectrum turns up below 2,500 Å. His data suggested a continued steep rise toward the shortest wavelengths observed (about 1,680 Å). More recent data (C. F. Lillie, unpublished), obtained with the ultraviolet spectrometer aboard Apollo 17, show that the spectrum turns down again below 2,000 Å. In fact, the relative intensity of the zodiacal light, normalised to the Sun, is now thought to be the same at about 1,680 Å and 4,250 Å. Thus, the conclusion of Kurt and Sunyaev that this component is negligible in the far ultraviolet is probably correct. In any case, a zodiacal light flux would certainly show a concentration toward the ecliptic. The zodiacal light, therefore, probably does not present any important limitation on the measurement of a cosmic far ultraviolet background.

Airglow

Probably the most important limitation on rocket observations of the diffuse far ultraviolet background is the contribution of line emission originating in the geocorona. By far the strongest of these lines is Lyman α $\lambda1,216$, whose intensity in the night sky is $\sim10^9$ photons (cm^2 s sr)$^{-1}$. No other lines have been observed, but it is possible that the OI λ1,304 triplet and OI λ1,356 will be significant at the flux levels under discussion here. If these lines have intensities of as little as 1 rayleigh, their contribution to the diffuse background measured with a typical photometer having a bandpass of about 200 Å is approximately 10^{-8} erg (cm^2 s sr Å)$^{-1}$. At present, such an intensity cannot be ruled out. Just as a small field of view is required to eliminate discrete spatial points (stars) which produce continuous spectral contributions, so is a narrow bandpass required to eliminate discrete spectral points (airglow lines) which produce a continuous spatial contribution, and attempts to observe the cosmic far ultraviolet background from rockets will require at least moderate resolution. Observations from high altitude satellites or interplanetary probes will not be limited in this way.

Observations

In spite of several attempts to measure the intensity of the diffuse ultraviolet background just longward and shortward of Lyman α, the subject remains in a state of confusion. Results of all previous measurements, displayed in Figure 1, range over two and a half orders of magnitude. The large range of the positive detections probably results from the large fields of view employed and the distribution of hot stars in the Galaxy.

The first attempt to measure the far ultraviolet background in the neighbourhood of Lyman α was made by Kurt and Sunyaev[2] using a detector aboard the interplanetary probe Venera-3. With a field of view of approximately 300 square degrees and a bandpass from λ1,225 to 1,340 Å, they detected no signal above that due to the cosmic ray background. Kurt and Sunyaev thus obtained an upper limit $I_\nu \lesssim 5 \times 10^{-22}$ erg (cm^2 s sr Hz)$^{-1}$ or $I_\lambda \lesssim 1.0 \times 10^{-9}$ erg (cm^2 s sr Å)$^{-1}$. This result is generally considered suspect, because Kurt and Sunyaev[20] later reported a detection, with the same instrument, of the hot stars near the galactic plane at an intensity which is two orders of magnitude below the theoretically expected value[13,21] and the value observed by Hayakawa, Yamashita, and Yoshioka[17] in the same direction.

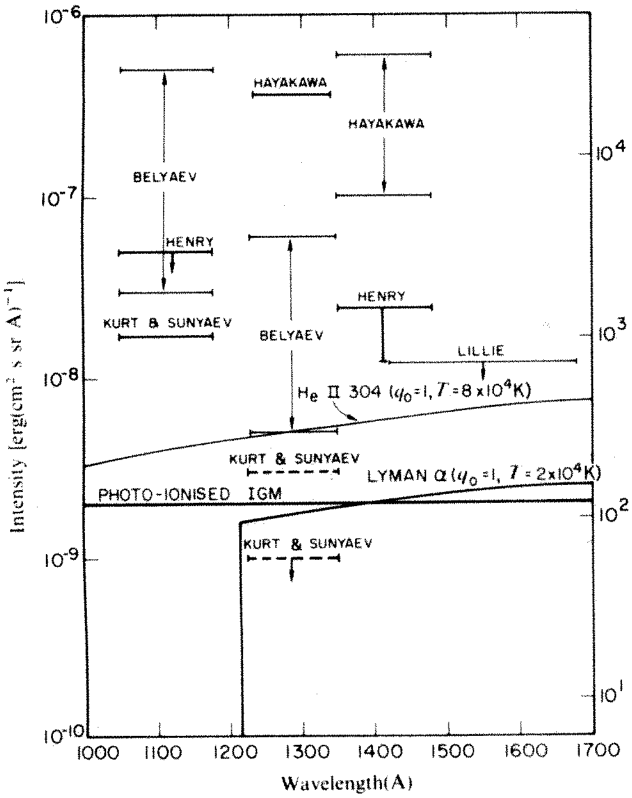

Fig. 1 Observed and predicted intensities of the diffuse far ultraviolet background. The measurements are discussed in the text. The lowest intensities, due to Kurt and Sunyaev, are contradicted by later results. The Lyman α flux from recombinations IGM with $q_0 = 1$ and $T = 2 \times 10^4$ K (assumed constant with Z) is shown. A similar flux due to He II 304 Å recombinations at large Z is also shown. Also presented is an estimate of the flux which is necessary to produce a sufficient degree of photoionisation in an IGM having the critical density. Local and Galactic components of diffuse light are probably weak enough at high latitude to allow detection of a cosmic flux of approx 10^{-9} erg (cm^2 s sr Å)$^{-1}$. A sensitivity at this level can be achieved with current instrumentation.

Another measurement in the region λλ1,225 to 1,340 Å was reported by Belyaev *et al.*[22] with an instrument aboard Venera-5 and Venera-6. These authors also measured the background in the range λλ1,050 to 1,180 Å. The fields of view were approximately 120 and 0.4 square degrees for the shorter and longer wavelengths, respectively. Intensities obtained in the λλ1,225 to 1,340 Å interval varied from about 2×10^{-8} to about 5×10^{-8} erg (cm^2 s sr Å)$^{-1}$ near the galactic plane and an upper limit of $I_\lambda \lesssim 5 \times 10^{-9}$ erg (cm^2 s sr Å)$^{-1}$ was obtained in an undetermined direction. The results were of very low statistical accuracy because of the small field of view, a rather small collecting area (about 10 cm^2), and a high cosmic-ray background. In the interval λλ1,050 to 1,180 Å, Belyaev *et al.* obtained intensities ranging over about 3×10^{-8} to 65×10^{-8} erg (cm^2 s sr Å)$^{-1}$. The authors attribute the much higher intensities observed in this band to the larger field of view.

Hayakawa, Yamashita and Yoshioka[17] have measured the background intensity in the range λλ1,350 to 1,480 Å with a detector whose field of view was 20 square degrees. Their results ranged from about 5×10^{-7} erg (cm^2 s sr Å)$^{-1}$ near the galactic plane smoothly down to about 1×10^{-7} erg (cm^2 s sr Å)$^{-1}$ at $b \gtrsim 40°$. These authors also obtained an upper limit to a possible extragalactic intensity of $\lesssim 1.7 \times 10^{-8}$ erg (cm^2 s sr Å)$^{-1}$ on the basis of an analysis of the latitude variation in terms of the scattering properties of interstellar grains. This analysis was subsequently revised[23] with the result that no information on the extragalactic component was obtained.

Using photometers aboard OAO-2, Lillie[24] has obtained an upper limit on the diffuse intensity at high galactic latitude in a 260 Å band centred at 1,550 Å. With the extremely small field of view (about 0.02 square degrees) of this instrument, the contribution due to hot stars was eliminated. Unfortunately Lillie observed no signal above the dark counting rate because of the low throughput of the instrument. His resultant upper limit was 1.2×10^{-8} erg (cm^2 s sr Å)$^{-1}$.

Henry[25] has reported measurements of the high latitude background in the range λλ1,050 to 1,180 Å and λλ1,350 to 1,475 Å, made with a field of view of approximately 100 square degrees. In the longer wavelength range, he obtained an intensity of about 2×10^{-8} erg (cm^2 s sr Å)$^{-1}$. Shortward of Lyman α, he obtained only the upper limit (I_λ 1,100 Å) $\lesssim 3 \times 10^{-8}$ erg (cm^2 s sr Å)$^{-1}$ since the measured signal was largely due to residual sensitivity of his detector to Lyman α radiation.

Intensities

It seems likely that most of the large range of intensities in Fig. 1 is due to the varying number of hot stars with galactic latitude. These are the main contributors to the intensity measured with a large field of view. In addition there is undoubtedly an important contribution from diffuse galactic light at low latitudes. Except for the results of Kurt and Sunyaev, the lowest intensities were obtained with the detectors having the smallest fields of view. These results were upper limits, and are limited by the sensitivity of the instruments. Unfortunately, these limits are not yet strong enough to place important constraints on the density of the IGM. A sensitivity of $\lesssim 10^{-9}$ erg (cm^2 s sr Å)$^{-1}$, achievable with current instrumentation, would provide an interesting limit. Local and galactic components of diffuse far ultraviolet radiation are probably weak enough at high galactic latitude to allow detection of a cosmic intensity of this magnitude.

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Primary structure of a mouse myeloma cell initiator transfer RNA

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The primary structure of cytoplasmic tRNA$_f^{Met}$ (the initiator tRNA) from mouse P3 myeloma cells is given and its most unusual features are discussed.

THE initiation step of mammalian protein synthesis involves a unique methonine-accepting transfer RNA. This initiator tRNA, which is usually denoted tRNA$_f^{Met}$ in respect of its ability in its charged form to function as a substrate for *E. coli* methionyl-tRNA$_f^{Met}$ transformylase, serves to donate the amino acid which is to form the N-terminus of the newly synthesised polypeptide[1-3]. In prokaryotes, as well as within the cell organelles of eukaryotes, N-formyl-methionyl-tRNA$_f^{Met}$ functions as the initiating species of tRNA, whereas in the cytoplasm of eukaryote cells no formylation of methionyl-tRNA$_f^{Met}$ occurs before it provides the first amino acid of nascent polypeptides. The methionyl-tRNA$_f^{Met}$ of both bacteria and higher organisms is distinguished from other aminoacyl-tRNAs engaged in protein synthesis by its inability to participate in polypeptide elongation and thereby provide its amino acid moiety for the internal positions of a protein amino acid sequence. Methionine residues which are not N-terminal in the protein are contributed by another methionine-accepting tRNA species, denoted tRNA$_m^{Met}$, which operates in protein chain elongation but not in initiation.

We have investigated the structure of a mammalian initiator tRNA in the hope that it might be possible to relate a special tRNA structure to the unique role played by this molecule in protein biosynthesis. We report here the primary structure of the cytoplasmic tRNA$_f^{Met}$ of mouse P3 myeloma cells and discuss the most unusual features of the nucleotide sequence. To date only two other complete mammalian tRNA sequences have been reported[4,5].

The ^{32}P-labelled mouse P3 myeloma tRNA$_f^{Met}$ was isolated from the cytoplasm of cells cultured in the presence of ^{32}P-phosphate[6] and was purified by reversed-phase chromotography on an RPC-5 column[7]. The structural similarity of tRNA$_f^{Met}$ isolated from different mammalian sources is discussed in the following paper by Simsek, Boisnard, Petrissant and RajBhandary[8]. Fingerprints of the oligonucleotides obtained on digestion of mouse myeloma tRNA$_f^{Met}$ with T$_1$ RNase and pancreatic RNase are reproduced in Fig. 1. A comparison of these fingerprints with those in the accompanying paper serves to illustrate the close similarity between the nucleotide sequences of mouse myeloma initiator tRNA and other mammalian tRNA$_f^{Met}$ species which have been investigated. Oligonucleotide sequencing was performed using the techniques developed by Sanger and coworkers for the structural analysis of ^{32}P-labelled RNA, as detailed by Barrell[9]. The purification of the myeloma initiator tRNA and the determination of its nucleotide sequence will be described in detail elsewhere.

The complete primary structure of mouse myeloma cell cytoplasmic tRNA$_f^{Met}$ is presented in Fig. 2*a* arranged in a clover leaf. We consider that the two most noteworthy features of the structure are the loop IV (or -G-T-Ψ-C-loop) sequence together with the unusual presence of a cytidine residue immediately preceding the anticodon in the anticodon loop. We have obtained no evidence for any structural heterogeneity of mammalian cytoplasmic initiator tRNA so there is apparently only a single tRNA$_f^{Met}$ present in the cytoplasmic fractions derived from the cell line which we have studied. This is in contrast to *Escherichia coli* which contains two tRNA$_f^{Met}$ sequences which differ in the nucleotide found at a single location[10].

The outstanding characteristic of the mammalian initiator tRNA structure is its considerable similarity to the sequence of yeast tRNA$_f^{Met}$ which was determined by Simsek and RajBhandary[11]. There is also considerable, although not quite so extensive, homology with the nucleotide sequence of *E. coli* tRNA$_f^{Met}$ (ref. 10). The similarity between the primary structures of tRNA$_f^{Met}$ isolated from *E. coli*, yeast and mouse myeloma cell cytoplasm is shown in Fig. 2*b*. The special role of initiator tRNA apparently imposes structural constraints such that tRNA$_f^{Met}$ nucleotide sequences have only diverged to a limited extent during evolution. Much of the tRNA$_f^{Met}$ sequence conservation occurs in the regions of the clover leaf where the nucleotide sequences of tRNAs which charge with different amino acids generally show the greatest variation[12]. All three molecules compared in Fig. 2*b* are substrates for both *E. coli* methionyl-tRNA synthetase and *E. coli* methionyl-tRNA transformylase. The tRNA$_f^{Met}$ structural features which are possibly involved in *E. coli* methionyl-tRNA synthetase recognition are discussed in the following paper[8]. The conserved regions of nucleotide sequence in Fig. 2*b* might reflect features of tRNA$_f^{Met}$ structure which are necessary for *E. coli* transformylase recognition.

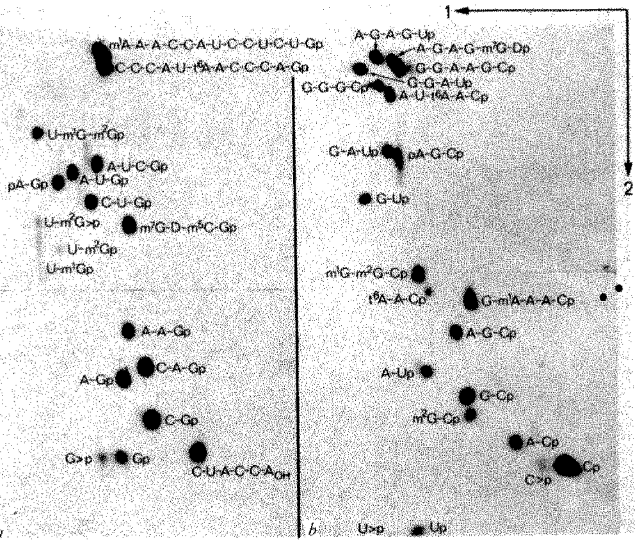

FIG. 1 Autoradiograms of two-dimensional fractionations of RNase digests of ^{32}P-labelled mouse myeloma tRNA$_f^{Met}$. Separations of the oligonucleotides in both a T$_1$ RNase digest (*a*) and a pancreatic ribonuclease digest (*b*) are shown. The first dimension (1) was electrophoresis at *p*H 3.5 on cellulose acetate and the second dimension (2) was electrophoresis on DEAE-cellulose paper in 7% formic acid.

The unusual unpaired base sequence -A-U-C-G-m^{1}A-A-A-, hitherto found only in the loop IV region of yeast initiator tRNA[11], is reproduced exactly in the mouse myeloma tRNA$_f^{Met}$ structure (Fig. 2*a*) and has apparently been conserved during eukaryote evolution. In general both yeast and mammalian tRNAs have pyrimidines as the first and last unpaired bases of loop IV of the tRNA clover leaf. As far as is known only in eukaryote initiator tRNAs are these pyrimidines replaced by adenosine residues[8]. In contrast *E. coli* tRNA$_f^{Met}$, the only prokaryotic initiator tRNA for which the sequence is known[10], contains a much more conventional sequence in loop IV (-T-Ψ-C-A-A-A-U-). It would be interesting to know whether the considerable differences in the loop IV sequences of prokaryote and eukaryote tRNA$_f^{Met}$ structures reflect a different mode of initiator tRNA binding to ribosomes in prokaryotes and eukaryotes. Recent evidence indicates that the initial binding of initiator tRNA to mammalian 40S ribosome subunits during the formation of initiation complexes may be independent of template[13,14] and therefore suggests that the initiation site on mammalian mRNA may be selected by the anticodon of tRNA$_f^{Met}$ bound to the subunit.

The mouse myeloma tRNA$_f^{Met}$ has cytosine immediately preceding the anticodon in place of the uracil residue hitherto universally found in this position in tRNA sequences. Virtually nothing is known at present about the molecular geometry of different anticodon loop primary structures, and therefore the significance of this base substitution remains unclear.

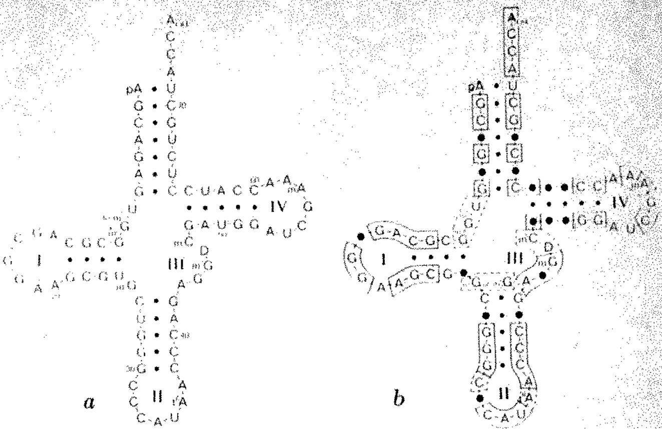

FIG. 2 *a*, The nucleotide sequence of mouse myeloma cell cytoplasmic initiator tRNA arranged in the clover leaf pattern. The Roman numerals I-IV identify the loops which are refered to in the text. *b*, An illustration of the similarities between the nucleotide sequences of the formylatable methionine-accepting tRNA species of mouse myeloma cell, yeast and *E. coli*. The clover leaf is drawn with those nucleotides which are common to both the myeloma and yeast tRNA$_f^{Met}$ sequences inserted in their appropriate positions. Regions of adjacent nucleotides common to the mammalian and *E. coli* initiator tRNA sequences are enclosed in boxes, the dotted lines indicating where these two sequences differ only with regard to base modification.

Since the methionine tRNA which participates in polypeptide elongation in mammalian cells, tRNA$_m^{Met}$, is aminoacylated *in vivo* by the same methionyl-tRNA synthetase as the initiator tRNA, it was pertinent to investigate whether a eukaryote tRNA$_m^{Met}$ would also have -A-U-C-G-m^{1}A-A-A- in loop IV. A preliminary analysis of the primary structures of the two major isoaccepting species of mouse myeloma cell tRNA$_m^{Met}$ has indicated that they possess different nucleotide sequences. The loop IV sequences of these two tRNAs are -T-Ψ-C-G-m^{1}A-A-C- and -T-Ψ-C-G-m^{1}A-U-C- (P. W. P., unpublished results), and therefore resemble those found in the majority of tRNAs of known primary structure (including *E. coli* tRNA$_f^{Met}$). This is a further indication that the loop IV of mammalian initiator tRNA may differ significantly in structure from the loop IV of mammalian tRNAs which function in polypeptide elongation.

Only information on the three-dimensional folding of the polynucleotide chains of tRNA molecules will indicate whether the constant features near the anticodon in initiator tRNAs reflect an anticodon loop structure which is unique to tRNA$_f^{Met}$ species. A special anticodon loop structure only found in initiator tRNAs might be a prerequisite for *E. coli* transformylase recognition and may also be responsible for the unexplained anomaly whereby tRNA$_f^{Met}$ of *E. coli*[15], yeast[16] and mouse ascites tumour cells[2] has the ability to recognise both the codons ApUpG and GpUpG and to thus exhibit code degeneracy or 'wobble'[17] at the third base (3′ end) of the anticodon. In contrast the tRNAs which function in polypeptide elongation produce the degeneracy of the genetic code by ambiguous reading of the template triplet or 'wobble' at the first base (5′ end) of the anticodon.

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Nucleotide sequence of rabbit liver and sheep mammary gland cytoplasmic initiator transfer RNAs

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The Nucleotide sequence of rabbit liver and sheep mammary gland cytoplasmic initiator tRNAs is identical to that of cytoplasmic initiator tRNA of mouse myeloma P3 cells.

THE first elucidation of the nucleotide sequence of a eukaryotic initiator tRNA was described recently[1]. It was shown that this yeast cytoplasmic tRNA possessed two striking features which distinguished it from all tRNAs of known sequence, including the *Escherichia coli* initiator tRNA. These were absence of the sequence G-T-ψ-C-G(A)-present in loop IV of most tRNAs and its replacement by G-A-U-C-G-; and presence of A as the last nucleoside of this loop instead of U or C as in other tRNAs. Further studies[2–5] have shown that both features are common also to mammalian cytoplasmic initiator tRNAs and that the entire sequence of loop IV of yeast and mammalian cytoplasmic initiator tRNAs are identical: A-U-C-G-m′A-A-A-. Studies on a plant cytoplasmic initiator tRNA have yielded essentially similar results except that the sequence of loop IV in this case is A-U*-C-G-m′A-A-A- (U*, modified U; Ghosh, Ghosh, G.P. and U.L.R., manuscript in preparation). These findings suggest that the two unusual features described above may be common to all eukaryotic cytoplasmic initiator tRNAs.

We summarise here the results of our work on the total nucleotide sequence of rabbit liver cytoplasmic initiator tRNA[6] and also provide evidence that the sequences of rabbit liver and sheep mammary gland[7] cytoplasmic initiator tRNAs may be identical. The sequence derived for the rabbit liver cytoplasmic initiator tRNA is identical to that deduced independently by Piper and Clark[8] for the corresponding tRNA of mouse P3 myeloma cells. Thus, the nucleotide sequences of cytoplasmic initiator tRNAs from three mammalian sources which have diverged significantly during evolution[9] have remained invariant. Details of this work will be described elsewhere.

Rabbit liver cytoplasmic initiator tRNA

The sequence of a fragment of thirty nucleotides obtained by cleavage of tRNA at the phosphodiester bond adjacent to m^7G has been reported previously[5]. The remainder of the tRNA molecule was sequenced by an analysis of fragments obtained by partial digestion of the tRNA with pancreatic RNase. The sequence is identical to that of mouse P3 myeloma cell initiator tRNA reported in the preceding paper.

Comparison of nucleotide sequences

The following lines of evidence strongly suggest that the sequences of rabbit liver and sheep mammary gland cytoplasmic initiator tRNAs are identical. (1) Modified nucleoside composition analyses using the technique of Randerath and Randerath[10] show that these two tRNAs contain the same modified nucleosides. The absence of m_2^2G which is present in the yeast cytoplasmic initiator tRNA and the presence instead of two mol of m^2G per mol of tRNA[2], one at G_{10} and the other at G_{25} is particularly noteworthy. (2) Fingerprints of complete T_1-RNase digests of these tRNAs are identical (Fig. 1). (3) Fingerprints of complete pancreatic RNase digests of these tRNAs are identical (Fig. 2). The corresponding spots from each of these fingerprints, Fig. 1 (*a* against *b*) and Fig. 2 (*a* against *b*), have been analysed further by 5′-end group analysis and partial digestion with snake venom phosphodiesterase. The patterns of radioactive spots obtained after partial snake venom phosphodiesterase treatment and electrophoresis on DEAE-cellulose paper[12] were identical for each of the corresponding spots. These results show that the series of fragments produced by T_1-RNase and by pancreatic RNase on rabbit liver and sheep mammary gland initiator tRNAs possess identical sequences.

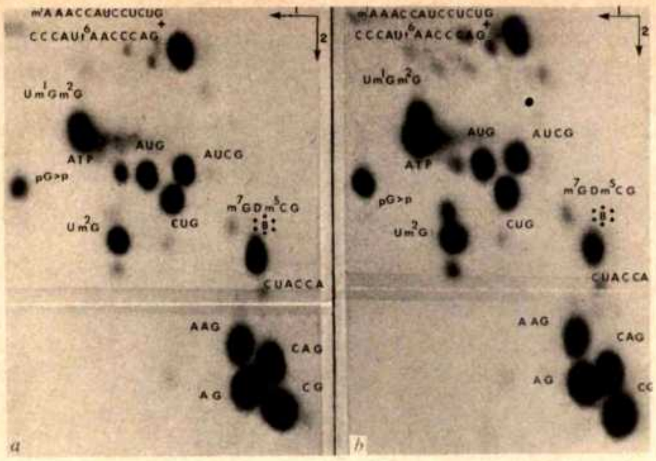

Fig. 1 Autoradiographs of 5′-^{32}P-labelled oligonucleotides obtained from T_1-RNase digests from rabbit liver (*a*) and sheep mammary gland (*b*) cytoplasmic initiator tRNAs. The procedure used was identical to that described before[2], except that 0.01 unit of *E. coli* alkaline phosphatase was used instead of 5 units. Except for pG-cyclic-p, all the oligonucleotides listed contain ^{32}P at the 5′ end and a 3′-hydroxyl group. The pattern obtained is almost identical to that obtained by Piper and Clark[8], except for the following: (1) the spot corresponding to pApGp in their fingerprint is present as pApG, (2) the spot corresponding to C-U-A-C-C-A_{OH} (ref. 8) is present in our fingerprints as pC-U-A-C-C-A_{OH}, and (3) Gp or G-cyclic-p is absent in our fingerprints. All of these differences arise because the *in vitro* labelling procedure involves removal of phosphomonoester groups present at either the 5′ and/or the 3′ end and their substitution by a ^{32}P at the 5′ end. The yield of m^7GDm^5CG has been consistently low (maximum obtained, 30%) in our experiments. This may be due to inefficient phosphorylation of the 5′-hydroxyl group in an oligonucleotide rich in modified nucleosides. In more recent fingerprints (ref. 5, see also Fig. 2), it has been possible to eliminate completely the spot due to ATP, which is present in this fingerprint. B surrounded by dots, blue dye marker.

With the work of Piper and Clark[8], the most striking result from our work is the observation that the cytoplasmic initiator tRNAs from three mammalian sources, those of mouse P3 myeloma cells, rabbit liver and sheep mammary gland, are identical. It would be interesting to determine whether these sequences have diverged further during evolution or whether they were conserved at an earlier stage of evolution, and studies along these lines are now in progress.

The total nucleotide sequence of four different methionine tRNAs which are aminoacylated by *E. coli* methionyl-tRNA synthetase with about the same K_m and V_{max} are now known. These include *E. coli* $tRNA_m^{Met}$ (ref. 13), *E. coli* $tRNA_f^{Met}$ (ref. 14) and yeast[15] and mammalian cytoplasmic initiator tRNAs[7]. Figure 3 shows a composite structure of these tRNAs. Except for the sequence of the anticodon loop, there is no extensive homology between these tRNAs. If the recognition of these tRNAs by *E. coli* methionyl-tRNA synthetase involves interactions with several different regions of the tRNA[16] and of specific nucleotides within these regions[17], the composite structure (Fig. 3) defines the maximum number of nucleotides which could be involved in this specific process. It is expected that sequence analyses on other methionine tRNAs which are recognised efficiently by *E. coli* methionyl-tRNA synthetase could narrow the extent of homology between these tRNAs still further[18] and provide the basis for a systematic approach to studying the problem of specific recognition between methionine tRNAs and *E. coli* methionyl-tRNA synthetase[19,20].

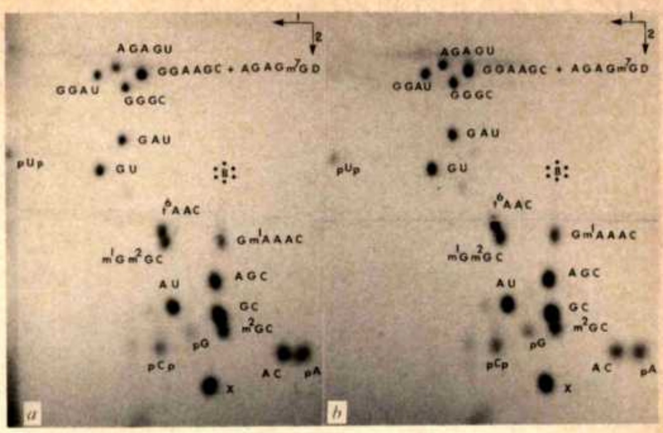

Fig. 2 Autoradiographs of 5′-^{32}P-labelled oligonucleotides obtained from pancreatic RNase digests of rabbit liver (*a*) and sheep mammary gland (*b*) cytoplasmic initiator tRNAs[5]. The pattern is similar to that obtained by Piper and Clark[8], except for differences expected on the basis of the *in vitro* labelling procedure used (see legend to Fig. 1). The relative positions of t^6AAC and m^1Gm^2GC in this fingerprint are reversed with respect to the fingerprint obtained by Piper and Clark[8]. This could be due either to minor differences between the nature of t^6A or to the presence of phosphomonoesters at the 5′ end of oligonucleotides on our fingerprints compared with 3′-phosphates in the oligonucleotides on the fingerprints obtained by Piper and Clark[8]. The radioactive spot X has been found in variable amounts in all of our fingerprints of pancreatic RNase digests of tRNAs—including non-initiator tRNAs. This probably arises from degradation of ATP by contaminating enzyme(s) in pancreatic RNase. Variable amounts of pA and pG have consistently been observed in fingerprints of pancreatic RNase digests of tRNA. We are now examining this phenomenon with regard to the possibility of traces of exonuclease contamination in the enzymes used. B surrounded by dots, blue dye marker.

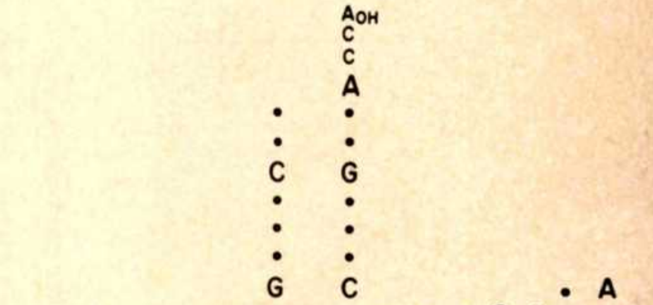

Fig. 3 Composite structure of methionine tRNAs. Nucleotides not common to these tRNAs are indicated by a dot. Nucleotides found in the same position in all tRNAs are indicated by small letter. Those uniquely common to the six methionine tRNAs are indicated by large letter.

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Cainozoic lacustrine stromatolites from hominid-bearing sediments East of Lake Rudolf, Kenya

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A new occurrence of algal stromatolites from Plio-Pleistocene lacustrine littoral sediments in the Eastern Rift, Kenya is described. These biolithites provide criteria for the interpretation of the environment of deposition of many of the hominids so far collected in the East Rudolf area.

THE documented stratigraphic history of the Lake Rudolf area, Kenya, now extends back 4.5 m.y. BP[1–11]. This record of terrestrial vertebrate evolution, sedimentary lithosomal variation and volcanic events during the Plio-Pleistocene now provides the chronology for the many hominid (*Australopithecus* and *Homo,* sensu Leakey[6–9]) remains recovered from the sediments of the Koobi Fora Formation in the East Rudolf Basin.

Events of lacustrine fluctuation are important to the chronology of much of the East Rudolf sedimentary terrane and characterise the Pliocene, Pleistocene and Holocene of the area[5,12,13]. The Lake Rudolph Basin, not always a closed basin evaporating pan, has had periodic connection with the Nile through an outlet (Sanderson's Gulf) north-west of the limits of the present lake[12]. As a result of these fluctuations, complex, interdigitated lithosomes of fluvial, deltaic and littoral lacustrine character are found throughout the area of sediment outcrop.

Detailed mapping of some of the hominid-yielding localities has demonstrated habitat association with nearby lacustrine strand environments[14]. Many of the sites are in turn overlain by transgressive lacustrine lithosomes. Characteristic of many of the basal facies of these transgressive lithosomes are extensive algal biolithites or stromatolites.

Previous investigations

Non-skeletal, organic sedimentary structures(stromatolites) produced by the mechanical accretion by filamentous algae of calcium carbonate, clay and other particles are quite common in the Plio-Pleistocene stratigraphic sequence of East Rudolf[2]. These structures usually occur as laminated muddy carbonates in which the cellular fabric of the original algae is not preserved.

The usefulness of shape as an environmental indicator has been discussed[15–33]. Three geometric forms of stromatolitic growth—mats, hemispheroids and spheroids—seem to be dominant. The environmental significance of each of these is so far unresolved, but seems to reflect various aspects of turbulence, turbidity, and/or desiccation in the littoral or supralittoral.

Various investigations of both ancient and modern lacustrine stromatolites[24–30] recognise the importance of turbulence in controlling growth form. Morphological zones in Recent stromatolites of the Great Salt Lake replicate and often enhance underlying substrate configuration[25]. Subsequent distribution and shape of individual stromatolites are controlled by erosional channel geometry developed perpendicular to the lake shore. Typical concentric growth is controlled by better aerated conditions on the leading edge of the colony Grove and Goudie[15,16] reported well-developed algal biolithites from various strand levels of Lake Stephanie, Ethiopia. Their observations on growth form, although preliminary, are very similar to the East Rudolf stromatolites described here.

East Rudolf algal biolithites

The sediments of the Koobi Fora Formation, Guomde Formation and Galana Boi beds have a lacustrine record which marks ancestral Lake Rudolf fluctuations. Typical proximal or near-shore to distal stromatolitic facies can be differentiated. Deep water stromatolites may exist but such examples are not abundant in Rudolf sediments.

I have mapped the East Rudolf collecting area 103 (Fig. 1, southern hatchered area) using several well-developed stromatolites, as markers of lacustrine transgression, to delineate transgressive/regressive stratigraphic cycles. The stromato-

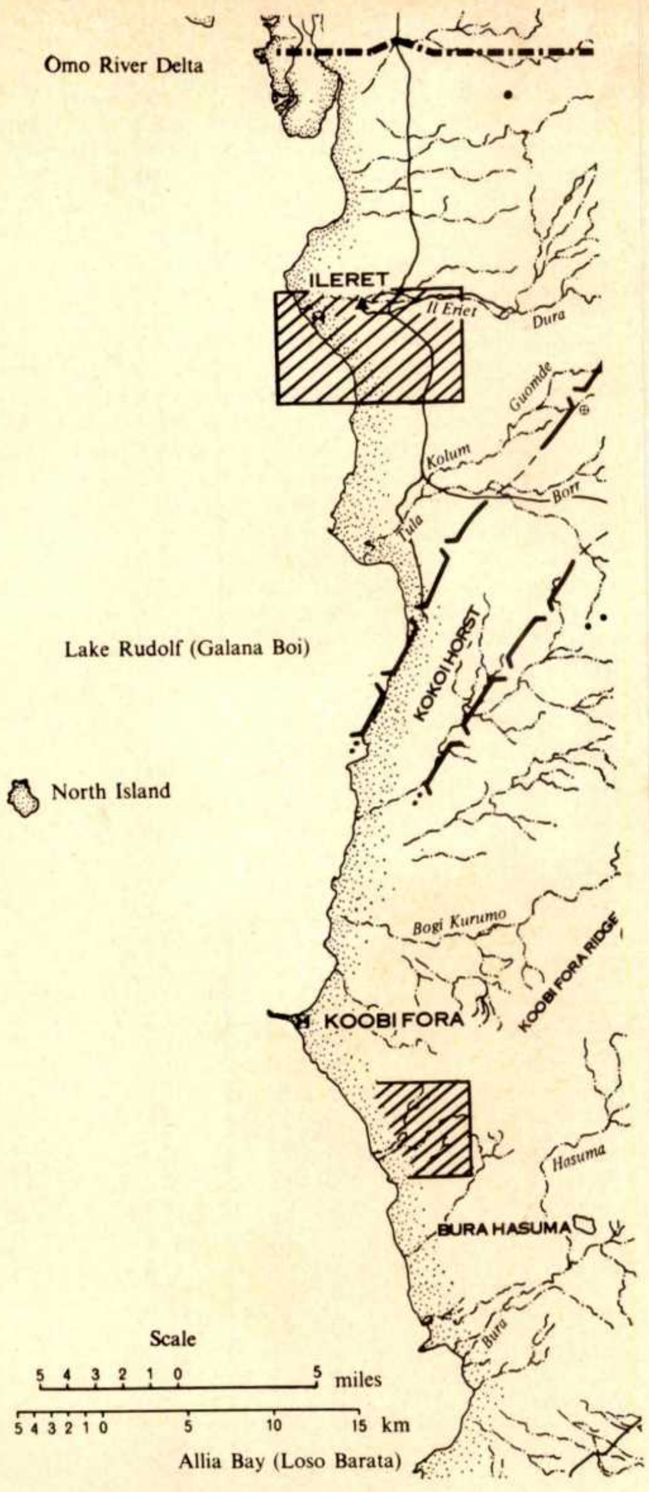

FIG. 1 Outline map of East Rudolf area showing localities of stromatolite development discussed in text.

lites which initiated these fluvial lacustrine intervals exhibit the most varied morphology and can be used to illustrate facies variability. Varied morphology gives rise to oncolites or concentrically stacked (laminated) spheroids (type SS-C; terminology after Logan, Rezek, and Ginsburg, ref. 17) and domes or vertically stacked (laminated) hemispheroids (type SH-V) to complexly compound forms (Figs 2 and 3). Analysis of all growth forms suggests open, solitary structures in more distal environments, with coalesced compound structures in more proximal environments. Several distinct facies can be recognised from the Upper Member of the Koobi Fora Formation:

Facies A. Polygonally desiccated algal mat containing a profuse gastropod epifauna (*Bellamya*). Individual polygons of thicknesses up to 3 cm have been noted. Polygonal diameters range from 10–15 cm. Throughout area 103, this facies is quite common and grades laterally into facies B (see Fig. 3*d*.)

Facies B. Concentric, smooth and nearly flat algal stromatolitic layers or mats. These are quite common in the central portion of area 103, and suggest fairly continuous, laterally extensive low energy environments of the shallow water lacustrine littoral. Epifauna is scare but associated vertebrate debris derived from local distributary sources and beach strand concentrations seem to predominate. In this facies, the stromalolites have resulted from lateral and vertical accretion over an irregular substrate which may include disarticulated allochthonous bone material and/or other allocthonous clastics (Fig. 3*c*).

Facies C. Discontinuous algal mats. These seem to be developed in fairly restricted shallow littoral environments and exhibit a variety of growth forms ranging from isolated

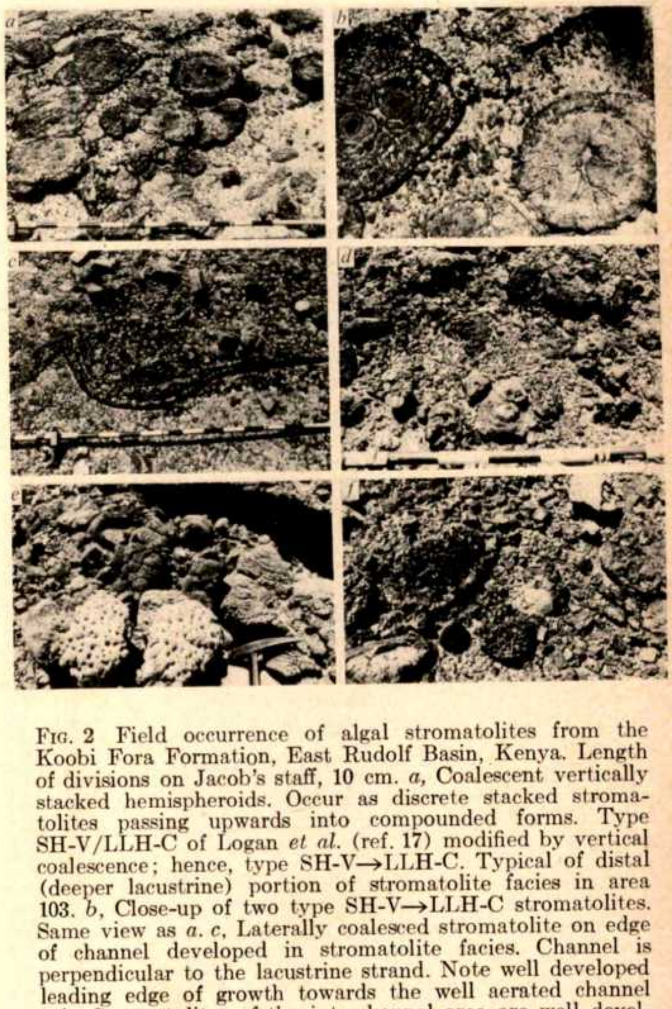

FIG. 2 Field occurrence of algal stromatolites from the Koobi Fora Formation, East Rudolf Basin, Kenya. Length of divisions on Jacob's staff, 10 cm. *a*, Coalescent vertically stacked hemispheroids. Occur as discrete stacked stromatolites passing upwards into compounded forms. Type SH-V/LLH-C of Logan *et al.* (ref. 17) modified by vertical coalescence; hence, type SH-V→LLH-C. Typical of distal (deeper lacustrine) portion of stromatolite facies in area 103. *b*, Close-up of two type SH-V→LLH-C stromatolites. Same view as *a*. *c*, Laterally coalesced stromatolite on edge of channel developed in stromatolite facies. Channel is perpendicular to the lacustrine strand. Note well developed leading edge of growth towards the well aerated channel axis. Stromatolites of the interchannel area are well developed type SH-V→LLH-C forms, all in natural growth position. Random stromatolites of type SH-V/LLH-C within the channels are commonly disturbed, and no longer in growth position. See below. *d*, Type SH-V, constant and variable basal radius stromatolites within channel areas. Note overturned domes; as many as 50% being disturbed. Channel width varies from 1-4 m across. Note relatively small initial growth surface preserved on some of the overturned forms. See *f*. *e*, Pitted type SH-V/LLH-C stromatolites from intra-channel area, area 103. Similar to pitted *Chlorellopsis* biolithites figured by Bradley (ref. 24, his plates 37 and 38). *f*, Overturned type SH-V/LLH-C stromatolites within channel area. Nuclear surface for stromatolite in lower left corner was shell of the bivalue *Mutela* sp. See *d*.

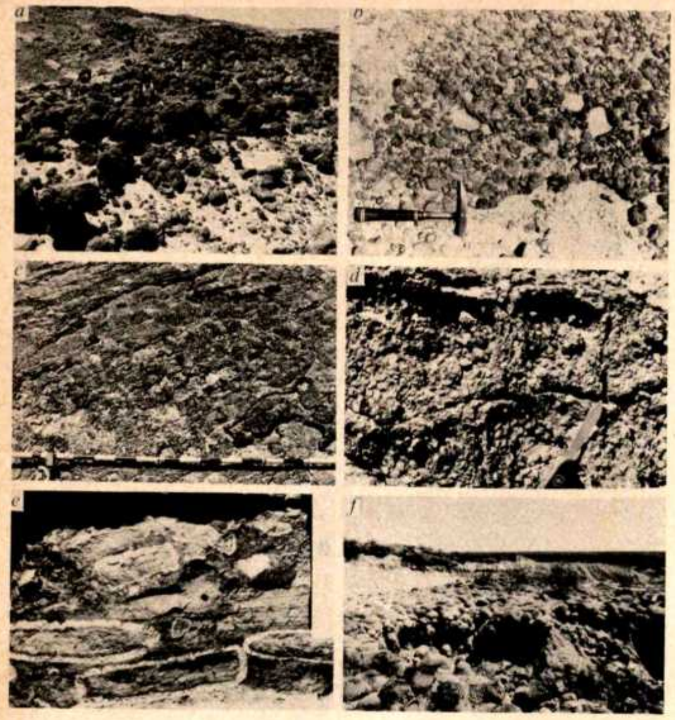

FIG. 3 Field occurrence of algal stromatolites from the Koobi Fora Formation and Galana Boi beds, East Rudolf Basin, Kenya. Length of divisions on Jacob's staff, 10 cm. *a*, Type SH-V stromatolites exhumed on extensive dipslope in central portion of area 103. Constitutes distal, turbulence-controlled facies of transgressive lacustrine lithosome. Overlying unit consists of an arenaceous mudstone which is easily winnowed from the intercolumnal areas. Stromatolites exhibit least amount of microstructure of any facies; composed of poorly sorted feldspatholithic sandstone. *b*, Type SS-C stromatolites (oncolites), area 103. Occur as extensive deposit in distal littoral facies. Nuclei are usually lithic clasts or bivalve (*Caelatura*) shells. *c*, Overview of typical stromatolitic biosome development showing general elongation of compound growth forms. Proximal, more coalesced forms to the right, out of picture. Distal, less coalesced forms to the left. Lineations are perpendicular to ancient shoreline. *d*, Desiccated algal mat facies. Profuse gastropod (*Bellamya*) epifauna occurs in this supralittoral facies. *e*, Mudstone clasts encased by stromatolitic carbonate. Note that some clasts are not encrusted evidencing mixed transport and residence time for these allochthonous shingles. *f*, Type SS-C stromatolites of the Galana Boi beds, Ileret. Largest forms may be 0.5 m in diameter. Unit grades laterally in size and nuclear material. Bench in distant background is limit of algal facies. *Etheria, Caelatura* are common nucleating bivalves. *Etheria*, the common river oyster, is the nucleus for most of the large forms in the foreground.

columnar structures to vertically stacked but laterally coalesced 'typical' stromatolitic structures. Logan[32] considered columnar growth a response to the upward accretion of stromatolitic laminae by increment entrapment of detrital material by filamentous algae producing a vertical stacking of hemispheroidal laminae (type SH-V). Numerous evidence from studies of marine intertidal stromatolites suggests that columnar growth may not be related to random isolated centres of stromatolitic development, but rather disarticulation of a once continuous algal mat by mechanical scouring of the substrate[21,32,33]. In this context, the large stromatolitic heads of area 103 which occur as irregular and laterally disarticulated, one-time cohesive algal surfaces have subsequently undergone coalescent algal growth.

Facies D. Compounded spheroidal and hemispheroidal stromatolites. These are generally laterally extensive, occurring in distal environmental facies throughout most of the Koobi Fora Formation. Surfaces upon which stromatolites nucleate are almost always lacustrine molluscs or lithic fragments (Fig. 2*a*, *b*).

Facies E. Concentrically stacked spheroidal stromatolites (oncolites). Throughout several intervals in area 103, and north at Ileret (Fig. 1, northern hatchered area) type SS spheroidal stromatolites (terminology from Logan, Rezak and Ginsburg, ref. 17) are found. These consist of stromatolotic coatings on the surface of shell debris, disarticulated vertebrate debris, and/or lithic fragments. Encrustations of less than 1 mm to complete spherical stromatolites 0.5 m in diameter are present (Fig. 3*g*, *f*).

Significance

Form and distribution of algal stromatolites in the East Rudolf terrane are significantly controlled by sediment input and subsequent reworking of the littoral lacustrine environment. As is the case with modern stromatolites of the shallow littoral of the Great Salt Lake[25], most stromatolites of East Rudolf seem to have responded to conditions of turbulence associated with erosion channels developed perpendicular to the ancestral Lake Rudolf strand. The regional variation, however, of the stromatolite-bearing strata is not known at present from the East Rudolf area. Mapping of microfacies variability is continuing because the lithosomal variation in East Rudolf sediments provide a wealth of palaeovironmental data. Detailed reconstruction of the East Rudolf palaeogeography for the interval represented by the Upper Member of the Koobi Fora Formation will be possible on the basis of these unique biogenic facies.

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Accumulation of fossil CO_2 in the atmosphere and in the sea

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An analysis from the chemist's point of view of the uptake of industrial produced carbon dioxide by the oceans reveals that serious environmental effects are not likely in the foreseeable future.

In recent months the threat posed by the accumulation of fossil CO_2 in the atmosphere has been given a new twist by the suggestion that, as a consequence, the surface layers of the ocean may become undersaturated with respect to calcium carbonate by the turn of the century[1]. Similar conclusions have been reached by other workers[2–5] although they did not comment on the possibly catastrophic biological consequences. Since the same predictions, with slightly differing time scales, are made using a number of mixing models[1,2,4]. I will not consider the relative merits of the various global treatments but will dwell instead on more mundane chemical matters.

Basically all the treatments[1–5] assume that the injection of fossil CO_2 into the ocean will remove carbonate ions according to the reaction

$$CO_2 \cdot H_2O + CO_3^{2-} \rightarrow 2HCO_3^- \qquad (1)$$

According to Broecker *et al.*[4] the sea will become undersaturated with respect to calcite when the carbonate content of the well mixed surface layers of the ocean has been reduced to 3.5×10^{-5} mol l^{-1}. Depending on the mixing model used this could occur well into the next century[4], by the year 2008 (ref. 1) or even earlier[2,3]. It is instructive to look at the chemistry of the seawater when this fateful day arrives.

A widely accepted mean recipe for seawater[6] gives the total concentrations of the carbonate $[CO_3^{2-}]_T$ and bicarbonate $[HCO_3^-]_T$ ions in seawater as 2.69×10^{-4} mol l^{-1} and 2.38×10^{-3} mol l^{-1} respectively in the early 1960s. These concentrations are related by the well known equation

$$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+ \qquad (2)$$

for which the stoichiometric constant K^*_{2C} may be defined as

$$\log K^*_{2C} = \log [CO_3^{2-}]_T/[HCO_3^-]_T - pH \qquad (3)$$

Hansson[7] has measured values of K^*_{2C} on the seawater ionic medium scale. Using his value at 25° C and 35‰ salinity equation (3) becomes

$$pH = \log [CO_3^{2-}]_T/[HCO_3^-]_T + 8.947 \qquad (4)$$

giving a *p*H of 8 for our initial seawater. The *p*H values here are also defined on the seawater ionic medium scale[8]. To reach the saturation level for calcite 2.34×10^{-4} mol l^{-1} of carbonate ions must be removed, resulting in the formation of 4.68×10^{-4} mol l^{-1} of bicarbonate according to reaction (1). In the saturated seawater then

$$pH = \log (3.5 \times 10^{-5}/2.85 \times 10^{-3}) + 8.947 = 7.04$$

suggesting even more fundamental biological implications. The carbon dioxide system in seawater is, however, contricted by a further restraint, that implied by the familiar equation

$$CO_2 \cdot H_2O \rightleftharpoons HCO_3^- + H^+ \qquad (5)$$

for which

$$\log K^*_{1C} = \log [HCO_3^-]_T - \log [CO_2 \cdot H_2O] - pH \qquad (6)$$

Again using Hansson's[7] value for K^*_{1C} at 25° C and 35‰ salinity and the final value of 2.85×10^{-3} mol l^{-1} for $[HCO_3^-]_T$ we find that

$$\log [CO_2 \cdot H_2O] = 5.857 - 2.545 - 7.04 = -3.728$$

According to Murray and Riley[9] $[CO_2 \cdot H_2O]$ is related to the partial pressure of carbon dioxide above the seawater (P_{CO_2}) at 25° C and 35‰ salinity by the equation,

$$[CO_2 \cdot H_2O]/P_{CO_2} = 289 \times 10^{-4}\ \text{mol}^{-1}\ \text{atm}^{-1} \qquad (7)$$

so that the partial pressure of carbon dioxide above our saturated seawater is 6.47×10^{-3} atm which is nearly twenty times the present value (3.33×10^{-4} atm) and an order of magnitude greater than the predicted partial pressure at the time saturation is achieved. Clearly something is wrong with the chemical hypothesis.

The error apparently arises because equation (1) is treated as a one way reaction rather than as an equilibrium so that it is assumed that every molecule of CO_2 taken in consumes a molecule of carbonate. If this were so no carbonate would exist in the oceans even now and all of the atmospheric carbon dioxide would have been used up long ago to give an oceanic sodium bicarbonate solution. The equilibrium equation can be formulated by subtracting equation (2) from equation (5). To understand the effects of fossil CO_2 on the oceanic mixed layer we must assess the contributions of the various components to the state of balance of this equilibrium.

The mixing models[1–5] themselves apply three constraints on the oceanic carbon dioxide system that must be considered in a chemical treatment. The concentration of carbon dioxide in the atmosphere at any point in time is fixed by the forward and backward projections of the current accumulation rates. The concentration of fossil carbon dioxide accumulated in the mixed layer of the oceans (and hence its total carbon dioxide content $C_T = [CO_2 \cdot H_2O] + [HCO_3^-]_T + [CO_3^{2-}]_T$) is also fixed at a level that depends on the assumptions made by the mixing model. Finally the carbon dioxide system in the mixed layer must be treated as an open system with free exchange of unhydrated CO_2 across the air-sea interface. I shall first make some rough predictions of the total concentrations of carbon dioxide in the atmosphere and in the mixed layer of the ocean at ten year intervals and will then apply these constraints to the oceanic carbon dioxide system.

The annual injection rate of carbon dioxide into the atmosphere has been calculated by Machta[10] and rather more pessimistic figures, on a decade basis, are given by Broecker *et al.*[4] Mixing models developed from these figures[1-4,10] indicate that only 60% of the carbon dioxide released into the atmosphere accumulates there. Most of the remaining 40% is removed by the oceans[4,10]. Assuming, along with Broecker *et al.*[4], that this partition will remain fairly constant if the current increase in production of 4.5% yr^{-1} continues, then values can be calculated for the atmospheric concentration of carbon dioxide (mol l^{-1}) at the end of each decade. I will take 1958 as my starting point using a mean world value[10] of 1.397×10^{-5} mol l^{-1} (313 p.p.m.) for the atmospheric concentration of carbon dioxide. For Machta's data[10] the total injection of carbon dioxide since 1958 is given by

$$\Delta CO_2 = (1.1/w_c V_a)\Sigma_i\, \delta_{i(M)}$$
$$= 2.333 \times 10^{-7}\, \Sigma_i\, \delta_{i(M)} \qquad (8)$$

where the annual increments ($\delta_{i(M)}$) are given by Machta[10] in units of 10^{16}g carbon per year, w_c is the atomic weight of carbon and V_a is the volume of the atmosphere[1] (3.93×10^{21} l). The factor 1.1 allows for Machta's corrections to his tabulated values[10]. The actual accumulation of carbon dioxide in the atmosphere since 1958 is taken as 0.6 ΔCO_2. Broecker *et al.*[4] give their data in units of 10^{16}g CO_2 per decade so that

$$\Delta CO_2 = (w_{CO_2} V_a)^{-1}\, \Sigma_i\, \delta_{i(B)}$$
$$= 5.783 \times 10^{-8}\, \Sigma_i\, \delta_{i(B)} \qquad (9)$$

The resulting change in C_T in the mixed layer of the oceans is given by the equation

$$\Delta C_T = (10^3 V_a/0.9Ad)y\, \Delta CO_2 \qquad (10)$$

where y is the fraction of the fossil CO_2 injection assimilated into the mixed layer, A is the surface area of the oceans[11] (3.61×10^{14} m^2) and d is the mean depth of the mixed layer[4] (70 m). The mixed layer is only formed over 90% of the ocean surface[1,4]. Substituting in the numerical values we find,

$$C_T = 2.650 \times 10^{-3} + 4.031 \times 10^{-5} y\, \Sigma_i\, \delta_{i(M)} \qquad (11)$$

or

$$C_T = 2.650 \times 10^{-3} + 9.990 \times 10^{-6} y\, \Sigma_i\, \delta_{i(B)} \qquad (12)$$

According to the model of Broecker *et al.*[4] only 5% of the total fossil CO_2 remains in the mixed layer so that $y = 0.05$. Machta[10], on the other hand, suggests that $y = 0.25$ and this is more in line with Fairhall's model[1]. The most pessimistic

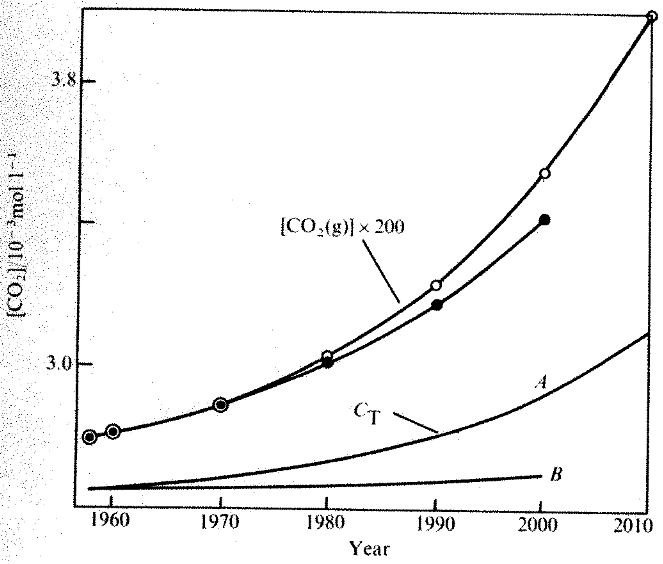

Fig. 1 Cumulative concentrations of carbon dioxide in the atmosphere (upper set) and in the oceanic mixed layer (lower set). ○, Using Broecker's injection figures[4]. ●, using Machta's injection figures[10]. *A* uses Broecker's[4] injection figures and $y = 0.25$ (refs 1, 10) (equation (12)) and *B* uses Machta's injection figures[10] and $y = 0.05$ (ref. 4) (equation (11)). These calculations represent respectively the highest and lowest predictions for the accumulation of CO_2 in the mixed layer.

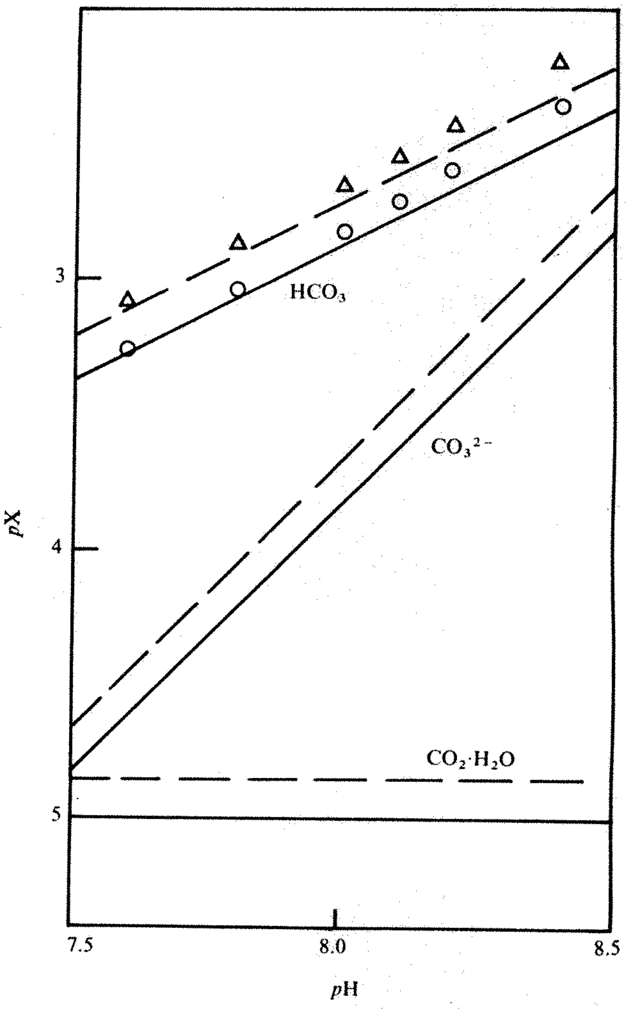

Fig. 2 Concentrations of the various components of the carbon dioxide system in the mixed layer plotted on a logarithmic basis against *p*H (equations (13) to (15)). ———, Concentrations in 1958; — — —, concentrations in 2010. Calculated total concentrations are also indicated (Δ, 2010; O,1958).

estimates of the accumulation of fossil CO_2 in the mixed layer then are obtained by using $y = 0.25$ in equation (12) (Fig. 1, curve *A*). By this estimate C_T will have reached 3.103×10^{-3} mol l^{-1} and $[CO_2(g)]$ $2.027 \times 10^{-}$[illegible] mol l^{-1} by the year 2010 (Fig. 1, curve *A*). This represents a more rapid accumulation of fossil CO_2 in the mixed layer than was assumed by Fairhall[1]. What will be the state of the oceanic carbon dioxide system at this time?

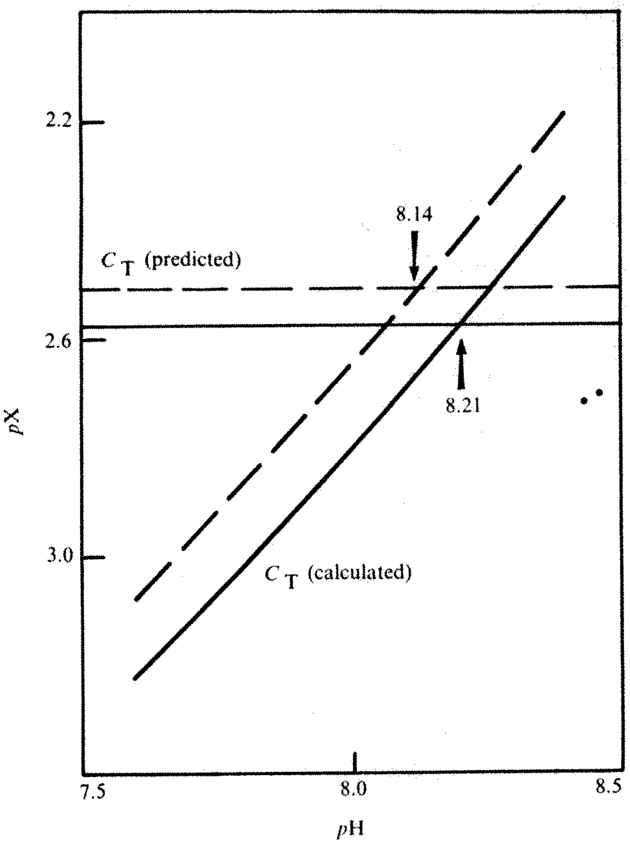

FIG. 3 Estimation of the equilibrium *p*H of seawater in the mixed layer in 1958, ——— and 2010 (— — —). The *p*H values (arrowed) are given by the points where the curves showing values of C_T calculated from equations (13) to (14) cross the horizontal lines representing the values of C_T predicted from equation (12) with $y = 0.25$.

To answer this question we must be able to estimate the total concentrations of carbon dioxide and of carbonate and bicarbonate ions in the mixed layer. These parameters together will fix the oceanic *p*H. The concentration of carbon dioxide in the mixed layer is related directly to the concentration of carbon dioxide in the atmosphere by equation (7). Since $[CO_2(g)]$ is fixed by the mixing model (Fig. 1, open circles) we can treat the oceanic system at any point in time as an open system[12] with the concentration of unionised carbon dioxide (mol l^{-1}) fixed by the equation

$$[CO_2 \cdot H_2O] = 0.647[CO_2(g)] \qquad (13)$$

at 25° C and total pressure 1 atm. The concentration of bicarbonate ions can be obtained by re-arranging equation (6) to give[12]

$$\log [HCO_3^-]_T = (\log [CO_2 \cdot H_2O] + \log K^*_{1C}) + p\text{H} \qquad (14)$$

Similarly by adding equations (3) and (6) and rearranging we find that

$$\log [CO_3^{2-}]_T = (\log [CO_2 \cdot H_2O] + \log K^*_{1C} + \log K^*_{2C}) + 2p\text{H} \qquad (15)$$

To fix the actual concentrations of these components (and hence the *p*H) we must use the second constraint applied by the mixing models, the known value of C_T at any point in time. A particularly simple analysis is possible if we plot the logarithm of the concentrations of each component (-log X = *p*X) against *p*H since the distribution of each species is then represented by a series of straight lines (Fig. 2). By summing these concentrations at regular *p*H intervals a cumulative curve showing $-\log C_T$ as a function of *p*H can be established (C_T (calculated), Fig. 3). The properties of the oceanic system will then be defined by the point at which this curve cuts the horizontal line corresponding to the value of C_T predicted by the mixing model (Fig. 3).

For the seawater at our arbitrary starting point in 1958 this gives $[CO_2 \cdot H_2O] = 9.05 \times 10^{-6}$ mol l^{-1}, $[HCO_3^-]_T = 2.20 \times 10^{-3}$ mol l^{-1}, and $[CO_3^{2-}]_T = 4.33 \times 10^{-4}$ mol l^{-1} at *p*H of 8.24 (Fig. 2). These results differ slightly from those calculated using equation (6) since the model used there[6] treats the ocean as a closed system. By the year 2010, according to the most pessimistic estimates, $[CO_2(g)]$ will have reached 2.027×10^{-5} mol l^{-1} so that $[CO_2 \cdot H_2O] = 1.312 \times 10^{-5}$ mol l^{-1}. Using this value and the predicted value of C_T (3.103×10^{-3} mol l^{-1}) the graphic method (Figs 2 and 3, dashed lines) predicts an equilibrium seawater with a *p*H of 8.16 and the following concentrations; $[HCO_3^-]_T = 2.65 \times 10^{-3}$ mol l^{-1} and $[CO_3^{2-}] = 4.36 \times 10^{-4}$ mol l^{-1}. The mixed layer is able to accomodate the fossil CO_2 injected by even the most pessimistic model without drastic changes in *p*H and without shifting the carbonate solubility equilibria appreciably.

The results of the calculations account, in a self-consistent manner, for the predicted atmospheric and oceanic concentrations. No account need be taken of the calcium carbonate equilibria since the mixed layer is supersaturated throughout, and is likely to remain so.

If the mixing models are correct in their predictions at C_T and $[CO_2(g)]$ then a gross supersaturation at the mixed layer with $[CO_2 \cdot H_2O]$ is required to produce carbonate dissolution.

This result is reassuring since it indicates that the assimilation of carbon dioxide in the mixed layer will not in itself give rise to any biological catastrophe[1]. It does, however, imply that the extra buffering mechanism for the absorption of carbon dioxide provided by the dissolution of the carbonate sediments[4] will not come into play until the bulk of the fossil CO_2 has penetrated the deep ocean. The consequences of man's profligate use of fossil fuel will therefore be apparent in the atmosphere for some time.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Detection of formaldehyde in external galaxies

FURTHER to our observations of OH in the edge-on spiral galaxies NGC253 and NGC4945 (ref. 1), we now report the detection of H_2CO absorption in both galaxies—the first evidence of this molecule in external galaxies.

The observations were made in early November 1973 with the Parkes 64-m telescope equipped with a 6-cm cryogenic parametric amplifier and a 512-channel autocorrelator. The latter was used in the total power mode. The correlator bandwidth was 10 MHz and the effective resolution was 19.5 kHz (equivalent to 1.2 km s^{-1} at the H_2CO rest frequency of 4,829.660 MHz). The beamwidth of the telescope was 4.2 arc min.

The H_2CO spectra obtained with the telescope directed towards the continuum maxima (coincident in position with the optical nuclei) of NGC253 and NGC4945 are shown in Figs 1 and 2 respectively. For comparison purposes OH and HI spectra obtained in similar directions are also shown; these have been discussed previously[1]. The H_2CO results represent about 6 h of integration on-source; an equal amount of time was spent at reference positions. Since no narrow spectral features exceeding the noise were found, the profiles shown have been smoothed to an effective resolution of 20 km s^{-1}.

For both galaxies the H_2CO absorption profiles are easily distinguishable. Although they are reasonably similar in shape and velocity coverage to their OH counterparts, the absorption at the more positive velocities is enhanced relative to the absorption at the lower velocities.

For NGC253 the H_2CO absorption, which has a maximum antenna temperature of 0.012 K, probably occurs against the

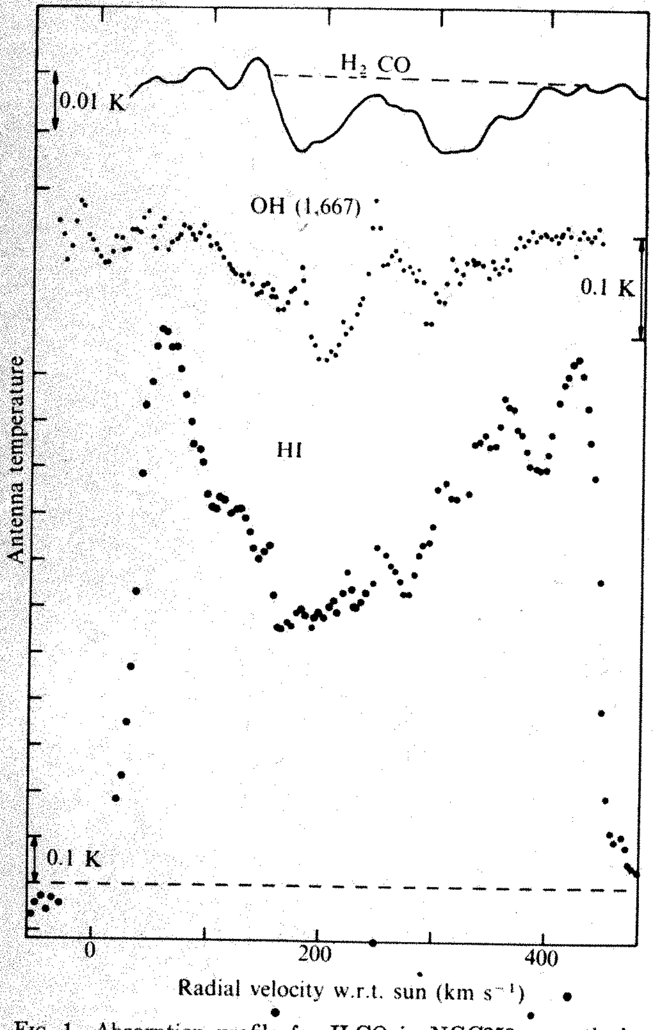

FIG. 1 Absorption profile for H_2CO in NGC253, smoothed to an effective resolution of 20 km s^{-1} in velocity. Comparison profiles for OH (1,667 MHz) and neutral hydrogen (1,420 MHz) are included.

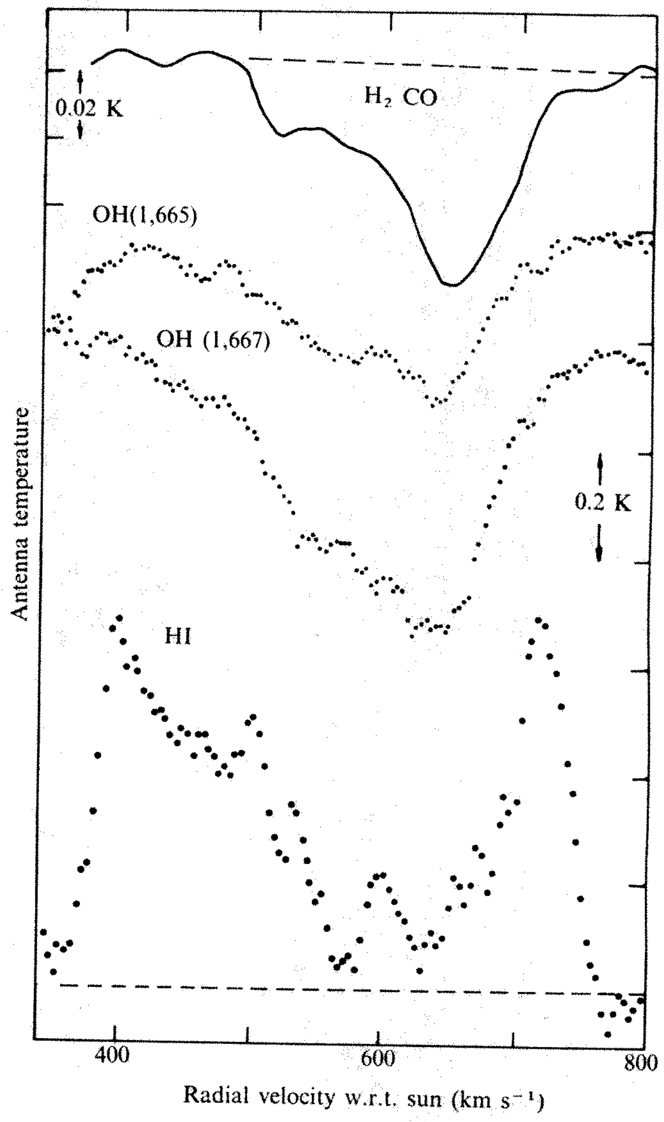

FIG. 2 Absorption profile for H_2CO in NGC4945, smoothed to an effective resolution of 20 km s^{-1} in velocity. Comparison profiles for OH (1,665 and 1,667 MHz) and neutral hydrogen (1,420 MHz) are included.

nuclear continuum component, as with the OH absorption. The maximum optical depth is about 0.02, considerably less than the corresponding value (0.07) for the OH absorption. There is no distinct feature that corresponds to the narrow-band OH emission near 250 km s^{-1}.

For NGC4945 spectra obtained at positions displaced from the nucleus by about ±3 arc min along the major axis were consistent with the absorption of an unresolved source coincident with the nucleus. If it is assumed that about 80% of the continuum flux at 6 cm originates in the nucleus, the antenna temperature of the peak absorption (0.066 K) corresponds to an optical depth of about 0.08. For the OH absorption the maximum optical depth is 0.3 (ref. 1). Compared with the molecular absorption in the Galaxy the optical depth of the H_2CO is unusually low—our (unpublished) observations of galactic sources suggest that the opacity of H_2CO generally exceeds that of OH when the optical depth of OH is greater than about 0.15.

It is worth noting that the relative enhancement of the H_2CO absorption at the more positive velocities, mentioned earlier, occurs also in the nuclear region of the Galaxy, where the ratio {optical depth (H_2CO):optical depth (OH)} is greater for the +40 km s^{-1} absorption than for the −130 km s^{-1}. The presence of absorption at radial velocities more positive than the systemic velocity in NGC253, in NGC4945, and in the Galaxy is most readily explained in terms of material moving towards the nucleus. It may be a common feature of the nuclear regions of spiral galaxies.

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Radio structure of the 'double quasar' 4C11.50

WAMPLER *et al.*[1] have recently announced the discovery of a close pair of optical quasars associated with the radio source 4C11.50. The brighter quasar (a) has a redshift of 0.436, whereas its companion (b), 4.8 arc s away in position angle 95°, has a much larger redshift of 1.901. The statistical probability of a chance coincidence is small and this has led to speculation that, despite the discordant redshifts and the lack of any visible connection, the objects may be physically associated. Hazard *et al.*[2] report unpublished observations from Cambridge and Westerbork which suggest that the radio structure is double, with one component coincident with quasar a. Here, we investigate further the alignment of the radio structure, seeking in particular any evidence of a radio connection between the two optical quasars.

The observations were obtained with radio link interferometers at Jodrell Bank during the period 1973 November–December. Details of the observing frequencies and baselines are given in Table 1.

TABLE 1 Parameters of Jodrell Bank interferometers used in the observations of 4C11.50

Home station	Out station	Observing frequency (MHz)	Baseline length (wavelengths)	Minimum lobe spacing (arc s)
Mark Ia	Defford	408	172,700	1.2
Mark II	Mark III	962	76,400	2.7
Mark Ia	Mark III	1666	132,300	1.6

The variation of fringe amplitude with hour angle for each of the three baselines is shown in Fig. 1. Parameters which describe the radio structure have been assigned using a model fitting technique which assumes components with elliptical gaussian distributions of surface brightness. Details of the best fitting models are given in Table 2 and the corresponding visibility curves are shown by the solid lines in Fig. 1. Although the separation and position angle of the double structure at 408 and 962 MHz are well defined,

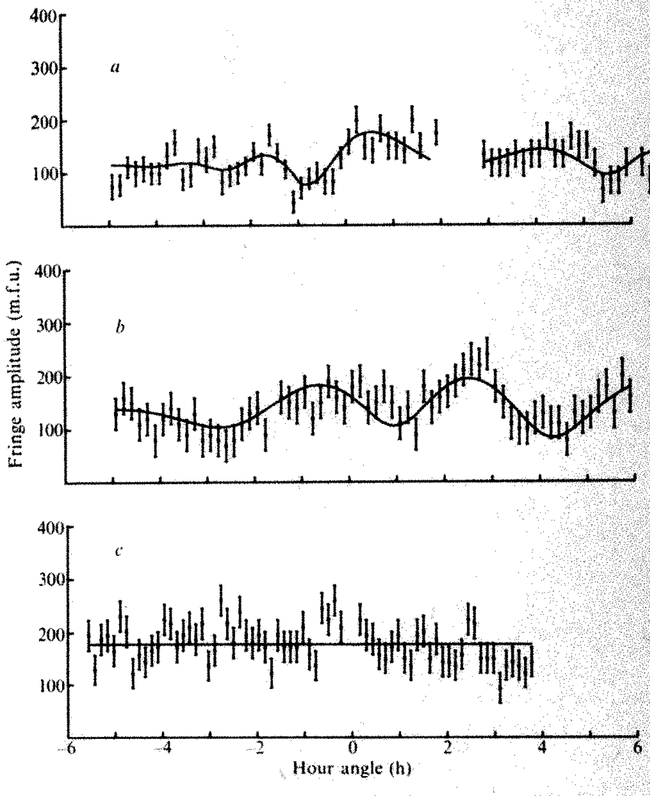

FIG. 1 Variation of fringe amplitude with hour angle at *a*, 408 MHz; *b*, 962 MHz; *c*, 1,666 MHz.

TABLE 2 Parameters of the best fitting models

			Component one		Component two		
Frequency (MHz)	Separation (arc s)	Position angle (degrees)	Flux density (m.f.u.)	Size (arc s)	Flux Density (m.f.u.)	Size (arc s)	Position angle of elongation (degrees)
408	14.0 ± 1.0	170 ± 2	115 ± 12	<0.3	65 ± 25	1.3 ± 0.4 x < 0.3	65 ± 25
962	12.8 ± 1.0	168 ± 2	146 ± 15	<0.6	77 ± 35	1.5 ± 0.7 x < 0.6	30 ± 40
1,666	—	—	179 ± 18	<0.4	—	—	—

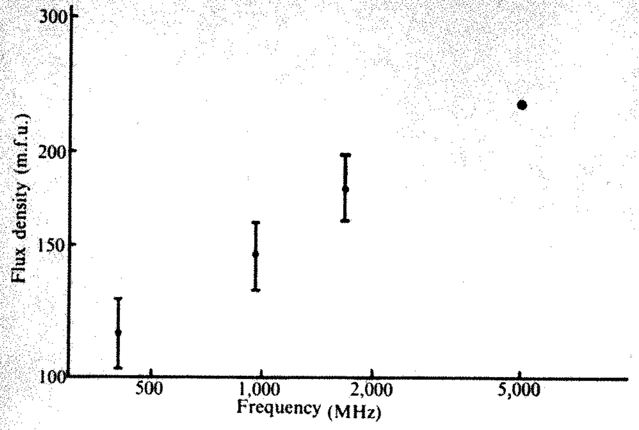

FIG. 2 Radio spectrum of the compact component coincident with 4C11.50a.

the parameters of the weaker component are more uncertain. At 1,666 MHz any variation of fringe amplitude is weak. Simple double models failed to provide a satisfactory fit to the data, which do not justify the fitting of any model more complicated than a single unresolved component.

The observations at 962 and 1,666 MHz were obtained using an improved local oscillator system developed by R. E. Spencer and R. S. Warwick (unpublished). At both frequencies the resulting phase stability over a period of several hours is sufficient to derive a position in right ascension for the more intense component. This position coincides, within our error of ±2 arc s, with the optical position of quasar a given by Wills *et al.*[3] The short term phase stability is not good enough to say whether the weaker component lies to the east or west of this position, and hence there is an ambiguity of 180° in the position angle of the double structure given in Table 2.

The stronger component is unresolved at all three frequencies with an angular diameter $\lesssim$0.5 arc s. The derived flux densities may therefore be used to construct a spectrum. This is shown in Fig. 2, where we have also included a measurement made with the Westerbork synthesis telescope at 5 GHz (R. D. Ekers, private communication). The radio spectrum is inverted, with a spectral index α (defined in the sense that $S \propto f^{\alpha}$) of +0.29. This combination of a compact structure and an inverted spectrum suggests that the component may well be variable at centimetre wavelengths.

At 408 MHz the flux density accounted for by our model is only 0.18 f.u., considerably less than the zero spacing flux density of 1.8 f.u. given by Hazard *et al.*[2] This discrepancy indicates that extended structure of low surface brightness is present, to which our high resolution interferometers are insensitive. Synthesis observations at lower spatial frequencies are needed to define such structure. The observations at 408 and 962 MHz show, however, that the compact component associated with quasar a has a resolved companion situated about 13 arc s away in position angle 170°. At 1,666 MHz, where the resolution is comparable with that at 408 MHz, any contribution from this component is less than 30 m.f.u., which implies that it has a normal radio spectrum. The angular structure and component spectra of 4C11.50 therefore show some similarity to those of the quasar 3C273. In the case of 4C11.50 however, the secondary component is elongated in a position angle somewhat different from that of the main double structure.

At all three frequencies the observations enable us to place an upper limit of 30 m.f.u. on any radio emission from compact structure associated with quasar b. Neither the axis of the double radio source, nor the elongation of the extended component is directed at quasar b. Thus the evidence on the radio structure of 4C11.50 presented in this paper suggests that there is no radio association between the two optical quasars.

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Airborne measurement of the temperature of the cosmic microwave background at 3.3 mm

THE 'primaeval fireball[1] model of the Universe' predicts a cosmic microwave background radiation field which has the spectral character of a blackbody source. The radiation arises from an initially very dense, very hot Universe, and has been cooled to around 2.7 K through the cosmological expansion. Previous ground-based radiometric measurements[2–6] at wavelengths as short as 3.3 mm are consistent with a 2.7 K blackbody, but most of these points are in the Rayleigh-Jeans tail of such a blackbody spectrum.

The short wavelength direct radiometric points are shown in Fig. 1. The expected curvature of the spectrum shown in Fig. 1 should be observable even at 3.3 mm, however, and both existing measurements[7,8] in this atmospheric window are inconsistent with a gray body or power law spectrum. They are consistent with one another and the blackbody model. Even though both experiments were performed at high altitude sites in an attempt to minimize atmospheric emission from H_2O and O_2, in no case was the atmospheric signal less than nine times the signal attributed to the background radiation. By carrying a radiometer above the tropopause, the H_2O and O_2 contribution is reduced to the point that the atmospheric emission is roughly equal to the cosmic signal.

Because the blackbody nature of this observed radiation is crucial to the validity of the big bang hypothesis and the departure of the spectrum from a power law can be checked at a wavelength of 3.3 mm, we attempted to obtain direct radiometric data free from large atmospheric effects by constructing a 3.3 mm Dicke radiometer designed specifically for mounting in a Lear Jet aircraft made available by the NASA Ames Research Center at Moffett Field, California. The instrument was carried above the tropopause on five flights in May and July of 1971; but only one flight was successful. During the course of that flight, we made 21 measurements of the background intensity at various zenith angles and report here a minimum variance weighted average of those points indicating a thermodynamic temperature of (2.48 ± 0.54) K.

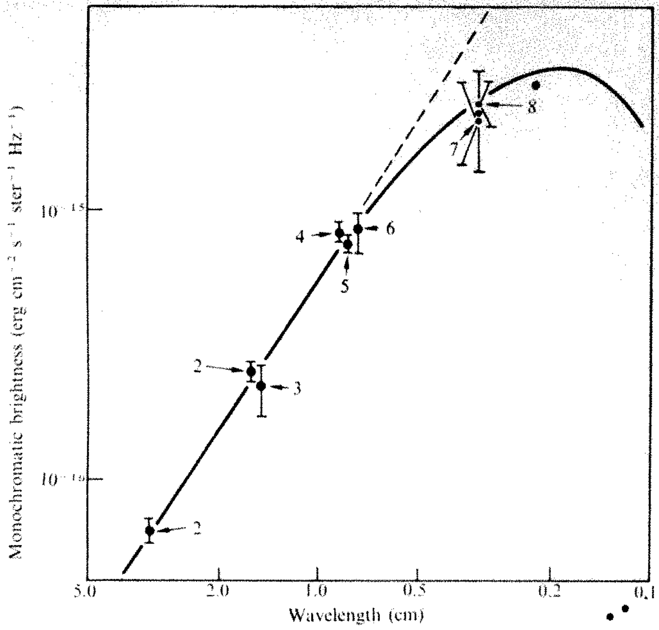

FIG. 1 Measurements of the cosmic background. Numbers indicate appropriate references. ———, 2.7 K blackbody; — — —, grey body.

The radiometer uses a balanced mixer with Schottky barrier diodes and a 500 to 1,000 MHz intermediate frequency. The overall noise figure of the radiometer was about 11dB, giving an r.m.s. temperature of ±0.1 K for an integration time of 1 s.

We paid particular attention to the stability of the secondary reference standard which was used to provide a constant radiation temperature at one port of the Dicke switch. A high quality microwave termination was immersed in boiling liquid helium to provide the necessary low temperature blackbody reference source. The temperature profile along the waveguide connecting the helium load to the switch was controlled by two additional fixed points, one at the boiling point of liquid nitrogen, the other at a closely regulated temperature of 30° C. Altogether, five critical waveguide sections were electronically temperature controlled and independently monitored. In addition, the entire r.f.-i.f. system was enclosed in an insulated box within which temperature-controlled air was circulated. An additional control system was used to keep the liquid helium level on the waveguide fixed despite changes in cabin attitude or altitude.

TABLE 1 Results of airborne measurements made with the 3.3 mm radiometer

Time	Sec θ	$T_{SKY} - T_{CL}$ (K)
1356	2.28	+0.64 ± 0.29
1358	1.70	−0.14 ± 0.29
1400	1.39	−0.02 ± 0.45
1401	1.70	+0.05 ± 0.37
1410	1.70	−0.25 ± 0.29
1411	1.29	+0.00 ± 0.27
1420	1.94	+1.12 ± 0.27
1426	1.14	+0.25 ± 0.26
1429	1.94	+1.13 ± 0.45

With the radiometer mounted in the plane, the radiation entering the antenna, T_{SKY}, is the sum of the background radiation, T_{BG}, and atmospheric radiation, T_{ATM}. This signal is compared at 1,000 Hz with the signal from the secondary reference standard. Just before and after the flight, the radiometer is calibrated by mounting it on a special stand outside the plane and viewing the primary reference standard with the antenna. The primary reference standard consists of a microwave absorber immersed in liquid helium; the conical taper of the antenna is continued into the dewar[7]. The signal entering the antenna during the calibration, T_{CL}, is composed of radiation from: (1) the cold absorber, T_{HE}; (2) the metal wall of the cold-load pipe, T_{WALL}; (3) the cold-load window, T_{WINDOW}; and (4) reflection of radiation back into the horn by the load, T_{CLR}. The Dicke switch compares the two signals T_{CL} and T_{SKY} in turn with the signal from the secondary reference standard and if one forms the difference $T_{SKY} - T_{CL}$, the switch asymmetry and the secondary reference signal are eliminated. The various other signals are measured in independent experiments, leaving a single unknown, the background temperature, T_{BG}, which is given by

$$T_{BG} = (T_{SKY} - T_{CL}) - T_{ATM} + T_{HE} + T_{WALL} + T_{WINDOW} + T_{CLR} \qquad (1)$$

By taking data with the aircraft maintaining several different constant bank angles, it was possible to obtain a fit to a simple atmospheric model. The term T_{ATM} in equation (1) is given by $T_0 \sec \theta$ is the zenith angle and $T_0 = (1.21 \pm 0.37)$ K was inferred from the fit. The last four terms in equation (1) were determined during the earlier ground-based experiment which used the same primary reference standard;[3] the contributions were found to be:

$$\begin{aligned} T_{WALL} &= 0.22 \pm 0.11 \text{ K} \\ T_{WINDOW} &= 0.27 \pm 0.04 \text{ K} \\ T_{CLR} &= 0.02 \pm 0.01 \text{ K} \\ T_{HE} &= 2.40 \pm 0.01 \text{ K} \end{aligned} \qquad (2)$$

During our flight, 32 sky measurements were made from an altitude of 49,000 feet on July 19, 1971; the results are summarised in Table 1. Visual inspection of the radiometer chart tracing indicated a rapid drift in the output between the points taken at 1411 and those at 1420. The origin of the drift has not been determined. The remaining 11 points were deemed unreliable and are not included in our determination of T_{BG}. A weighted average of the first 21 values of T_{BG} gives

$$T_{BG} = (0.92 \pm 0.40) \text{ K} \qquad (3)$$

where the error is the standard deviation in the mean (weighted by the inverse variance of $T_{SKY} - T_{CL}$ for each point). If we assume that the radiation is from a blackbody, the thermodynamic temperature which would produce this antenna temperature is

$$\tau_{BG} = \left(2.48 \pm {0.50 \atop 0.54}\right) \text{ K} \quad \text{at } \lambda = 3.3 \text{ mm} \qquad (4)$$

The previous results at this wavelength are:

$$\begin{aligned} \tau_{BG} &= \left(2.46 \pm {0.40 \atop 0.44}\right) \text{ K} \quad \text{(ref. 7)} \\ \tau_{BG} &= (2.61 \pm 0.25) \text{ K} \quad \text{(ref. 8).} \end{aligned} \qquad (5)$$

The new result is consistent with these, but most importantly, the atmospheric background subtracted from our result is only one seventh of that substracted from the measurements.

A greybody source would produce an antenna temperature 2.7 times our result.

We thank Robert Cameron and the staff of the NASA Ames Research Center for their hospitality and cooperation during the course of our observations. Mr J. Burton provided

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Upper bound on the electric charge of a black hole

THROUGH an analysis of reversible transformations of black holes, Christodoulou and Ruffini[1] have shown that the energy of a rotating, charged black hole is given by

$$E^2 = (m_{ir} + Q^2/4m_{ir})^2 + L^2/4m_{ir} \qquad (1)$$

where m_{ir} is the irreducible mass of the black hole, Q its charge and L its angular momentum. They have also shown[1] that for a given irreducible mass, the charge and angular momentum must satisfy the condition

$$L^2/4m_{ir}^2 + Q^4/16m_{ir}^4 \leq 1 \qquad (2)$$

Once the equality is satisfied, no more charge or angular momentum can be added to the black hole without increasing m_{ir}.

A different expression for an upper bound on the black hole's charge can be derived by considering a loss of charge by pair production. If the electric field of the black hole creates an electron-positron pair outside the event horizon, one of these particles can enter the black hole and the other can travel to infinity. This process will occur only if the decrease in total energy of the black hole for a unit change in charge is sufficient to produce a free electron (or positron). The following discussion is limited to negatively charged black holes so that the ingoing particles are positrons. For positively charged black holes, one interchanges electrons and positrons.

In order to compute the energy of the ejected electron, we differentiate equation 1 to obtain

$$\begin{aligned}\Delta E = (1/E)(m_{ir} + Q^2/4m_{ir})(Q/2m_{ir})\,\Delta Q \\ + \Delta L\,\partial E/\partial L + \partial E/\partial m_{ir}\,\Delta m_{ir}\end{aligned} \qquad (3)$$

ΔE is the change in the energy of the black hole and ΔQ the change in its charge. For $\Delta L = 0$ and $\Delta Q = Q_e$, the electron charge, equation (3) yields

$$fm_e = (1/E)(m_{ir} + Q^2/4m_{ir})QQ_e/2m_{ir} \qquad (4)$$

where m_e is the electron charge and f gives a measure of the kinetic energy of the ejected electron and any increase in m_{ir} associated with the process. The latter effect, an increase in m_{ir}, requires some explanation. It is possible that some of the energy obtained by decreasing the electric charge increases the irreducible mass; however, equations (3) and (4) assume that m_{ir} is constant. For $\Delta m_{ir} > 0$, the energy change calculated with equation (3) must exceed the final energy of the ejected particle in order to increase m_{ir}. The energy change is thus formally treated in two steps, a change in charge alone followed by a change in m_{ir} alone. The energy associated with the change in m_{ir} is included in the analysis by properly choosing f. Equation (4) will of course be useful only if we can obtain a minimum value of f for which particle ejection is possible because this value of f will determine the upper limit on the charge of the black hole.

If the ejection process can just occur, the electron will reach infinity with zero kinetic energy. Thus, a minimum value of f larger than 1 corresponds to an increase of m_{ir}. Since we can certainly create the positron with a minimum total energy (kinetic + gravitational) of m_e the minimum value of f is less than 2. The $f = 2$ case corresponds to the creation of a free electron and free positron, each with zero kinetic energy at infinity, one of which falls back into the black hole. It is quite reasonable, however, to believe that $f = 1$. This latter statement follows from Christodoulou and Ruffini's result[1] (based on a mechanism proposed by Penrose[2]) which shows that it is possible to change the charge and angular momentum of a black hole such that m_{ir} does not change. An appropriate value of the positron's energy and momentum state will allow this to happen, and this value can be obtained in pair production with some non-zero probability density.

For $f = 1$, equation (4) yields an upper limit on the charge of a black hole of

$$1 \geq (1/E)(m_{ir} + Q^2/4m_{ir})Q/2m_{ir}Q_e/m_e. \qquad (5)$$

Because $Q_e/m_e = 2 \times 10^{21}$ in gravitational units, Q should be small compared to m_{ir}. Thus,

$$1 \gtrsim (Q/2E)(Q_e/m_e) \qquad (6)$$

The large magnitude[3] of Q_e/m_e yields an upper limit on the electric charge much lower than the limit obtained by Christodoulou and Ruffini[1] except for the case approaching maximal angular momentum, where equation (2) predicts that $Q = 0$. For $L = 0$, Christodoulou and Ruffini obtain $Q \leq 2m_{ir}$, whereas equation (5) yield $Q \leq 2m_{ir}(m_e/Q_e) = 2m_{ir}(5 \times 10^{-22})$, a difference of twenty-two orders of magnitude.

In order to understand the difference between these limits, we proceed by computing the rate of charge loss. We first note that essentially all of the electrons formed by the field should escape because equation (6) is the same equation one obtains in Newtonian gravitation. Thus, the rate of charge loss can be calculated by computing the rate of pair production in the field. Schwinger[3] has computed the rate Γ of electron-positron pair production in an electrostatic field as

$$\Gamma = (\alpha^2/\hbar\pi^2)\epsilon^2\left[\sum_{n=1}^{\infty} n^{-2} \exp\,(-n\pi m_e^2/q_e\epsilon\hbar)\right] \qquad (7)$$

where α is the fine structure constant; ϵ is the electric field, and q_e, the electron charge. For this rate to be significant, $\pi m_e^2/(q_e\epsilon\hbar) \lesssim 1$. If the electric field is estimated as $Q/4M^2$ (in gravitational units), one obtains for the zero angular momentum case)

$$1 \lesssim (\lambda/2\pi M)(Q_e/m_e)(Q/2M) \qquad (8)$$

where λ is the Compton wavelength of the electron $\hbar/(m_e c)$.

The case of a 1 solar mass black hole yields $1 \lesssim 1.73 \times 10^5$ $Q/2M$ for a significant rate. The lowest value of Q for which this rate is significant is thus in this case approximately 10^5 lower than the upper limit computed by Christodoulou and Ruffini. Furthermore, for $Q = 2M$, one can use equation (7) to estimate the rate of charge loss. A conservative estimate is obtained with $\Gamma \sim \alpha^2\epsilon^2/\hbar\ \pi^2$. This value of Γ must be multiplied by a volume which we estimate as R^3 and a time dilation factor which is no worse than $\bar{\lambda}/2M$ (this is the gravitational time dilation term at a distance $\bar{\lambda}$ from the Schwarzschild radius). This yields a rate for $Q = 2M$ of at least $\alpha^2c^4/(\hbar G) = 10^{60}$ electrons s^{-1} provided only that the black hole mass M allows equation (8) to be satisfied with $Q \leq 2M$.

Thus, the difference between the upper limits occurs because equation (6) is a weaker statement than equation (2). The upper bound given by equation (6) can be temporarily violated: charge can be added, however the added charge will be lost if the black hole is left alone. Equation (2), on the other hand, can never be violated. We further note that as the charge becomes larger [for value of Q above the upper limit of equation (6)] more energy is available for the process and f therefore increases above its minimum value. This increase in energy can be distributed arbitrarily between the electron and positron. The electron may have non-zero kinetic energy, and m_{I}, can increase. The phase space restrictions on the ejection process are then less than for smaller charges. This, together with the increase in the pair production rate with increasing charge, causes the increase in the charge ejection rate.

The following physical picture then emerges: starting with an uncharged black hole, one can add charge until the inequality in equation (6) is satisfied. Beyond this point, charge may still be added: however, a net inflow of charge is necessary to balance the loss of charge by the mechanism proposed in this paper. The charge may then be increased until the absolute bound given by equation (2) is reached.

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Meteoritic magnetite as a cosmothermometer

Latimer[1] and Urey[2] first investigated the oxidation states of iron in the dust phase of the solar nebula, demonstrating for the reaction

$$\tfrac{3}{4}\ \mathrm{Fe(s)} + \mathrm{H_2O(g)} \rightleftarrows \tfrac{1}{4}\ \mathrm{Fe_3O_4(s)} + \mathrm{H_2(g)} \qquad (1)$$

that the equilibrium constant $K = \mathrm{H_2/H_2O}$ is determined by the relative abundance of hydrogen and oxygen in the solar nebula. The equilibrium constant is further related to the standard free energy change, ΔG°, by the well known thermodynamic relation

$$\Delta G^\circ = -RT \ln K \qquad (2)$$

Thus, with ΔG° obtained from thermodynamic data and K determined by the solar $\mathrm{H_2/H_2O}$ ratio, the equilibrium temperature of ~400 K is calculated.

On the basis of the above thermodynamic reasoning, the presence of magnetite in carbonaceous chondrites has frequently been cited as a cosmothermometer to infer accretion temperatures of $\lesssim 400$ K (refs 3–5). The equilibrium temperature determined from equations (1) and (2), although providing a 'go-no- go' criterion for the formation of magnetite from the oxidation of metallic iron, yields no information regarding the potential sensitivity of that reaction to local, possibly non-ideal, conditions. In this respect it is instructive to consider the separate equilibria involved in equation (1) which are related by the oxygen fugacity, $f_{\mathrm{O_2}}$. Independent of whether metoritic magnetite formed from the oxidation of metallic iron as proposed by Urey[2] or from the oxidation of troilite (J. M. H., unpublished), no loss of generality is expected from this approach as the formation and subsequent stability of magnetite in the early Solar System depends on the prevailing oxygen fugacity.

If we assume in an atmosphere of solar composition that the oxygen fugacity is governed by the gas phase reaction

$$\mathrm{H_2(g)} + \tfrac{1}{2}\ \mathrm{O_2(g)} \rightleftarrows \mathrm{H_2O(g)} \qquad (3)$$

we can calculate the oxygen fugacity as a function of temperature with the expression

$$-\log_{10}(f_{\mathrm{O_2}}) = -2\Delta G^\circ{}_f/2.3RT - 2\log_{10}(\mathrm{H_2O/H_2}) \qquad (4)$$

where the ratio $\mathrm{H_2O/H_2}$ is determined from cosmic abundance tables[6] and $\Delta G^\circ{}_f$ is taken from thermodynamic data. We can further calculate the oxygen fugacity equilibria for the reaction

$$3\mathrm{Fe(s)} + 2\ \mathrm{O_2(g)} \rightleftarrows \mathrm{Fe_3O_4(s)}. \qquad (5)$$

with the expression

$$-\log_{10}(f_{\mathrm{O_2}}) = -\Delta G^\circ{}_f/2(2.3)RT \qquad (6)$$

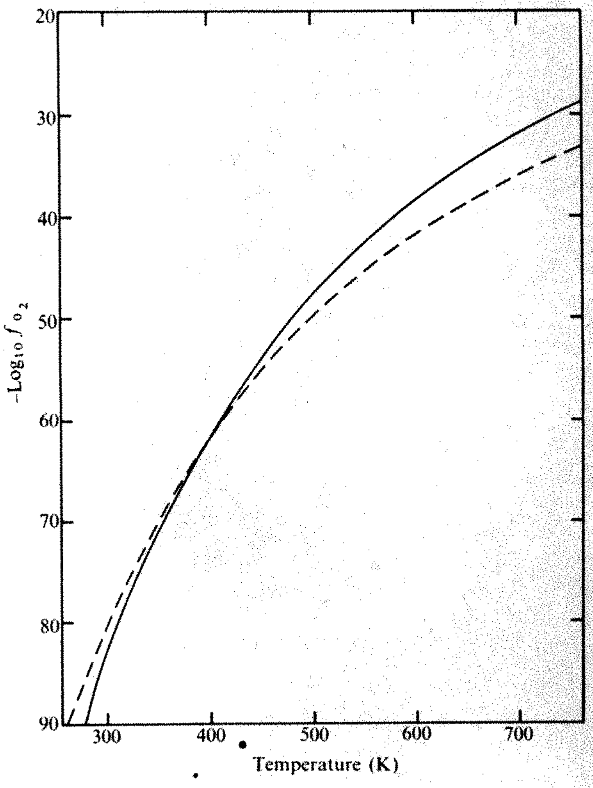

FIG. 1 Fe-Fe$_3$O$_4$ oxygen fugacity stability fields and the oxygen fugacity expected as a function of temperature in an atmosphere of solar composition. —, Fe-Fe$_3$O$_4$ oxygen fugacity equilibria line. The magnetite stability field lies above this line, metallic iron below. – – –, oxygen fugacity in an atmosphere of solar composition.

For temperatures under consideration here, FeO equilibria can safely be ignored.

The results of these calculations are shown in Fig. 1. For temperatures below ~400 K the oxygen fugacity as calculated for an atmosphere of solar composition lies within the magnetite stability region. This result is expected as equation (1) actually represents the combination of equations (3) and (5). The significance of these calculations, however, is demonstrated by the near coincidence of the two curves shown in Fig. 1. The Fe-Fe$_3$O$_4$ oxygen fugacity equilibria over a temperature range of several hundred degrees lies quite close to the oxygen fugacity expected in an atmosphere of solar composition. The expected oxygen fugacities as calculated are ideal. In the early Solar System only mild local conditions (such as gas phase reactions involving carbon or sulphur) need have occurred to have created oxygen fugacities which were either more oxidizing or more reducing than those calculated. Recognising that the formation and subsequent stability of magnetite is likely to be quite sensitive to local redox conditions over a wide range of temperatures, it seems inappropriate to use the presence of magnetite in meteorites as a cosmothermometer to infer accretion temperatures of $\leq$ 400 K.

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Radio emission from R Aquarii

R AQUARII has been known as a variable star since its discovery by Harding in 1811. Although R Aquarii is usually referred to as a long-period variable it has been described as a symbiotic nova by Merrill (ref. 1, page 84) and Payne-Gaposhkin[2]. Photographic evidence discussed below indicates that a nova outburst occurred approximately 600 yr ago. Prompted by the nova-like radio outbursts from Cygnus X-3 we started a search for radio emission from some of the eruptive variable stars. Here we report the detection of variable radio emission from R Aquarii.

The initial detection was made on April 23, 1973 at 10.5 GHz using the 46 m telescope of the Algonquin Radio Observatory. The measured flux density was 95 ± 10 m.f.u. (1 m.f.u. = 10^{-29} W m^{-2} Hz^{-1}). On May 19 the star was again observed at 10.5 GHz and the flux density recorded with 22 ± 10 m.f.u. The observations were obtained using the nodding technique[3] which uses a main and reference beam separated by 8 arc min in zenith angle. Both observations were made at the same hour angle so that the uncertainty in the measured flux density due to confusion in the two reference beam positions would be the same in each case. At this frequency the principal confusing sources are HII regions in the galactic plane. In view of the large galactic latitude of R Aquarii ($b^{\rm II} = -71°$) confusion effects are probably insignificant. The telescope was pointed on the optical position for R Aquarii within 15 arc s on the basis of the telescope pointing tests carried out on both occasions. The half-power beamwidth of the telescope is 2.8 arc min at 10.5 GHz.

Further observations were obtained using the Greenbank three-element interferometer at 2,695 and 8,085 MHz during two subsequent periods in June and August 1973. On both occasions a radio source was detected at the two frequencies coincident with the optical position given in the Smithsonian Astrophysical Observatory star catalogue. These results are given in Table 1. The observations for the two periods were combined to obtain synthesis maps of R Aquarii at both frequencies for all 300 m spacings up to 2,400 m. The synthesised beams at the two frequencies are identical except for a factor of three in size. The synthesised beam at 8,085 MHz is shown in Fig. 1 and the 8,085 and 2,695 maps of R Aquarii are given in Figs 2 and 3 respectively. R Aquarii appears to be unresolved at 8,085 MHz which places an upper limit on the source size of ≈1 arc s.

TABLE 1 Position and flux densities for R Aquarii obtained with the Greenbank-three-element interferometer

	RA (1950)		Dec. (1950)	
Radio position	23 h 41 min 14.16 s ± 0.05 s		−15°33′41.5″ ± 2″	
SAO catalog position	23 h 41 min 14.18 s		−15°33′41.79″	
Date	Interferometer spacings (units of 100 m)	Flux density m.f.u. 2,695 MHz	Flux density m.f.u. 8,085 MHz	Spectral index α
June 20–24	24–21–3	12 ± 2	9 ± 2	−0.3 ± 0.6
August	24–18–15–12–9–6	8 ± 1	7 ± 1	−0.2 ± 0.4

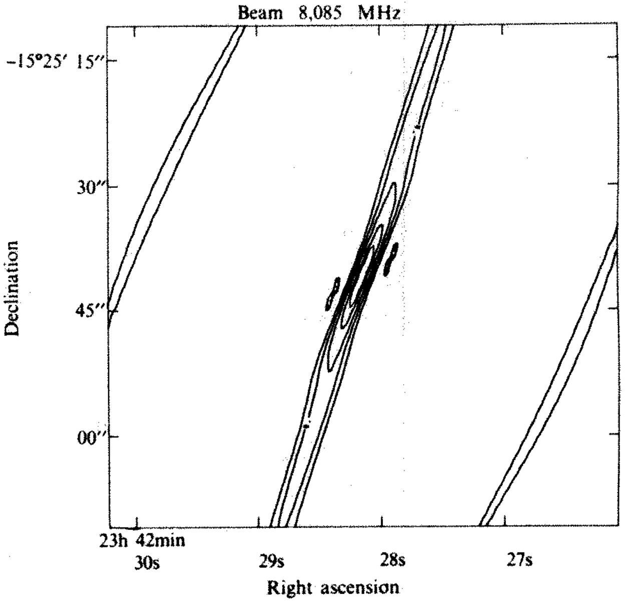

FIG. 1 The synthesised beam at 8,085 MHz obtained with the Green Bank Interferometer. A map of a single unresolved source with a high signal-to-noise ratio should look identical to the beam pattern. Epoch of coordinates given is 1973.6. Field of view 1 ×1 arc min.

No significant short term variability was detected during the interferometer observations and the flux density measurements at 10,500, 8,085 and 2,695 MHz are consistent with a slow decay in the flux density of R Aquarii throughout

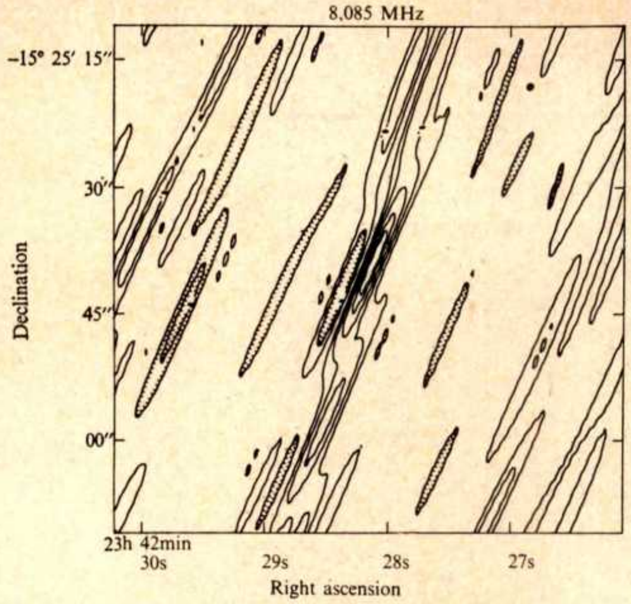

FIG. 2 Map of R Aquarii at 8,085 MHz with single-to-noise ratio of approximately 7:1. Hatched contours represent negative side-lobes. Field of view 1×1 arc min.

the entire period of observations from April to August 1973. The spectral index α as defined by the relationship S (flux density) $\propto \nu^{\alpha}$ is not significantly different from the value expected ($\alpha \approx -0.1$) for optically thin thermal bremsstrahlung radiation.

According to Merrill (ref. 1, page 84) the optical spectrum of R Aquarii shows evidence for both a red and blue component as well as emission lines characteristic of gaseous nebulae. The red component which is usually the stronger of the two is an M7e long period variable of period 387 d with an apparent magnitude range from 6.7 to 11.6. The spectrum of the blue component exhibits high excitation lines of hydrogen, helium and ionised iron. Merrill also points out that the brightness of the blue component has varied in an irregular manner. Although most of the time at eleventh magnitude or fainter it has been as bright as eighth magnitude. During 1931–33, when the blue component was especially bright, the Me variable exhibited a succession of abnormally low maxima. At this time the spectrum of the blue component was comparable to that of a Bep star.

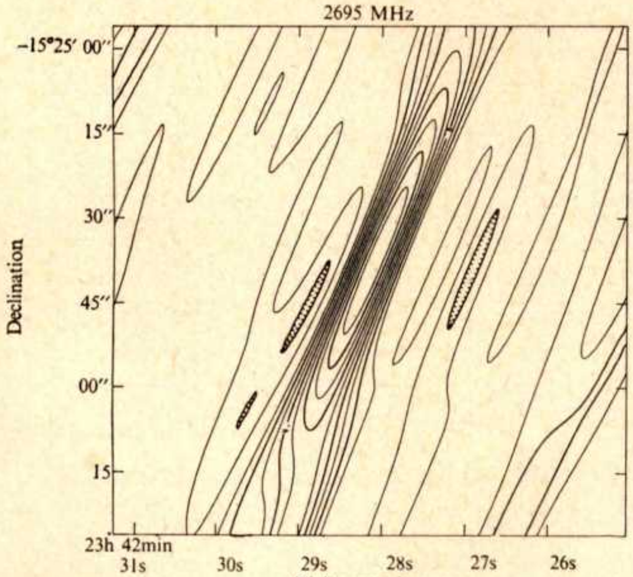

FIG. 3 Map of R Aquarii at 2,695 MHz with signal-to-noise ratio of approximately 9:1. Field of view 1.5 × 1.5 arc min.

The evidence suggesting that the blue component is an ex-nova comes from photographs revealing two interesting formations of faint nebular matter surrounding the star (see Fig. 4). The first photograph of R Aquarii was taken in 1921 by Lampland[4]. A summary of the chief characteristics of the nebulosity[5] is given below.

The most conspicuous feature is the lens shaped structure formed by two intersecting arcs in which the star is centrally and symmetrically placed. The position angle of the major axis of the arcs is about 90° and the greatest extent of the faint structure is a little more than 2 arc min. The inner nebulosity which is approximately 15 arc s in angular extent appears to be variable in position or brightness or both.

The outer nebulosity, although exhibiting no variations in brightness, was detected by Hubble to be expanding. The expansion, which is compatible with a nova-like outburst, was confirmed by Baade[6]. A pair of plates separated by a 16 yr time interval indicated that the nebulosity was ejected 600 yr ago, on the assumption of uniform expansion. Spectroscopic observations indicated an expansion velocity of 80–100 km s^{-1}, thus placing R Aquarii at a distance of 260 pc (ref. 6). This distance is consistent with the distance derived from the observed maximum visual magnitude of the long period variable.

It is clear from the photograph that R Aquarii is imbedded in a cloud of ionised gas with an angular extent of ~15 arc s. The general appearance of the inner nebulosity suggests that it was formed by the ejection of material from R Aquarii. A possible explanation of the radio emission is that it is optically thin thermal bremsstrahlung radiation from a denser central cloud of ejected matter surrounding the binary system and having an angular extent of <1 arc s (corresponding to a linear extent of 4 × 10^{15} cm). The variable radio emission could then be explained by a sudden ejection of matter by the blue component. The existence of the expanding outer nebulosity and general appearance of the inner nebulosity provides ample evidence for the ability of R Aquarii to eject matter.

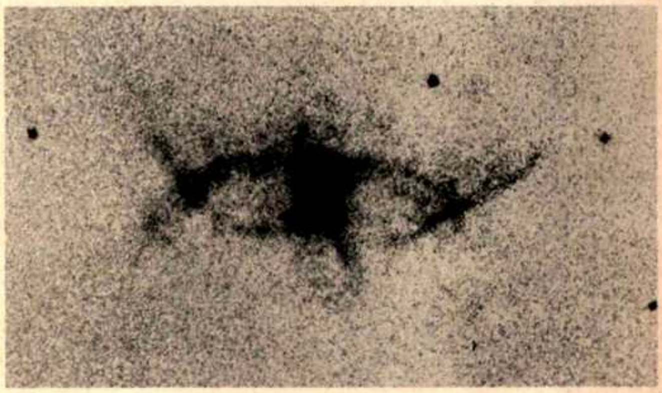

FIG. 4 Photograph of R Aquarii by E. P. Hubble with the Mount Wilson 100 inch reflector, October 12, 1939; exposure 1 h 30 m^{1}.

As one test of this model we can calculate a lower limit on the angular size of the central cloud required to explain the radio emission and compare this with the observational upper limit of 1 arc s. The lower limit is obtained from the following argument. For a thermal source of angular size θ the maximum possible flux density at the frequency ν is reached when the source is optically thick at this frequency

$$S = (2kT_e\nu^2/c^2)\theta^2 \qquad (1)$$

where T_e = electron temperature and k = Boltzmann's constant. From a knowledge of the optically thick value of S, equation (1) can be used to derive the product of $\theta^2 T_e$. If at the lowest frequency of observation the source is known to be optically thin then the observed flux density will be less than the optically thick value and the application of equation (1) will lead to a lower limit for $\theta^2 T_e$. For our observations of June and August a limit on θ of ≥ 0.5 arc s is obtained assuming $T_e = 10^4$ K. Since this value is less than the observational upper limit of 1 arc s the proposed model is consistent with our observations. In this respect observations at 1,420 MHz with the Westerbork interferometer would be extremely valuable for on the basis of this model we would predict that the central cloud should be optically thick at 1,420 MHz. Finally from the observed value of the optically thin flux density of the central cloud, the condition that $0.5'' < \theta < 1''$ and the known distance to R Aquarii of 260 pc we obtain limits for the r.m.s. electron density of $6 \times 10^4 < N_e < 2 \times 10^5$ cm^{-3} and limits on the mass of ionised gas of $3 \times 10^{-6} < M < 10^{-5} M_\odot$.

In addition to being a very interesting optical and radio source, R Aquarii has a large infrared excess thought to be produced by a dust cloud. At 11.5 μm the infrared flux density of R Aquarii[8] is 1.85×10^{-23} W m^{-2} Hz^{-1}. The ratio of the dust cloud emission peak to the cool star continuum is typical of other Mira variables such as o Cet and X Cyg[8] so that presumably the dust cloud is associated with the red component of R Aquarii.

The dates of the radio observations fall close to the expected minimum in the light curve of the red component of R Aquarii. From the optical data of the American Association of Variable Star Observers, kindly supplied to us by Margaret Mayall, there is no evidence of unusual optical behaviour immediately prior to or after the radio observations. Unfortunately there is an absence of optical data during April and May. Further observations are planned to examine the relationship between the radio and optical behaviour of R Aquarii.

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Fluctuations in oil flow before and after earthquakes

INTEREST has been growing recently in geophysical phenomena which occur near the time of earthquake events. We have been observing the flow of oil from wells in the Gulf of Suez and have noticed that remarkable fluctuations in flow occurred near the time of certain nearby earthquake events. The Gulf of Suez is considered to be an opening tensile region with Sinai moving away from the African continent[1]. The earthquakes occurred close to the bifurcation point of the Gulf of Suez and Eilat and the wells are about 100 km north from this point in the Gulf of Suez (Fig. 1, insert). The oil was driven up by natural pore pressure and was not pumped. Measurements of oil flow were made once a month. Figure 1 shows that from about January 1968 to June 1969, and from about April 1971 to July 1972, large fluctuations occurred in oil outflow. Also, within these periods and in particular towards the end, several moderate earthquakes occurred. In the first period there was an event on March 31, 1969 (magnitude

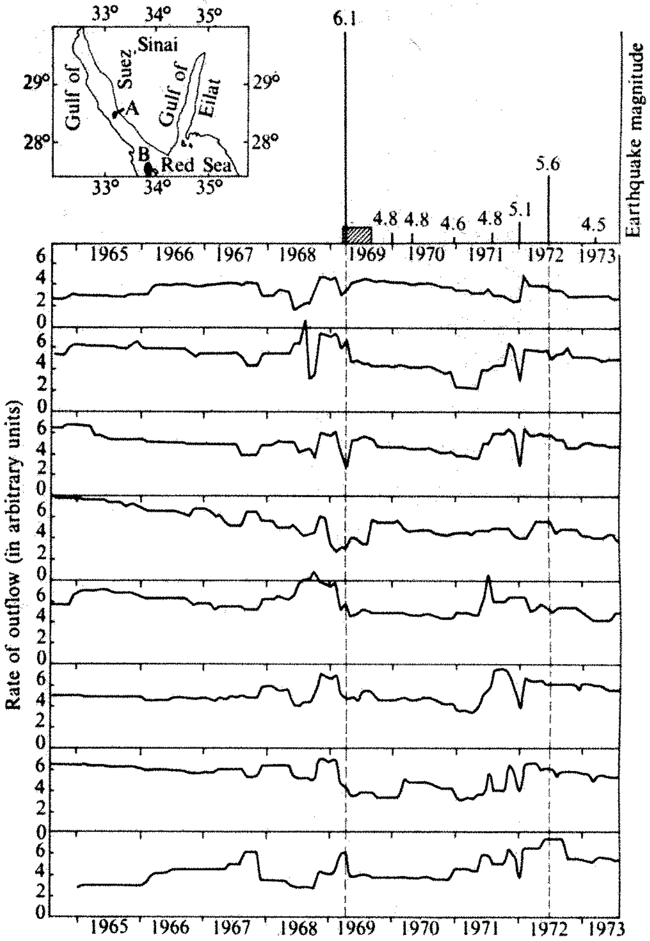

FIG. 1 Measurements of oil flow from wells in the Gulf of Suez, July 1964 to July 1973. Earthquakes are indicated above together with their magnitude. The shaded region under the 6.1 magnitude earthquake indicates foreshocks. Note the similarity of time function starting before the 1969 earthquake (magnitude 6.1) and the 1972 earthquakes (magnitudes 5.1 and 5.6). Inset, Map of Sinai region; *A*, Location of oil wells; *B*, Location of earthquakes.

6.1) plus a few foreshocks and many aftershocks reaching a maximum magnitude of 5.0. In the second period there were events on July 8, 1971 (magnitude 4.8), January 12, 1972 (5.1) and June 28, 1972 (5.6). Except for these periods the oil outflow was on the whole more stable and the earthquakes that did occur were of smaller magnitude, the largest ones being of magnitude 4.8 on December 30, 1969 and April 28, 1970. (No earthquakes occurred from January 1964 to February 1969.) The choke openings at the tops of the pipes were varied from time to time but after examination it seems to us that these variations did not cause the observed fluctuations.

If the simultaneous occurrence of flow fluctuations and moderate earthquakes is not coincidental, what is the possible connection between them? Pre-seismic deformations seem to occur over fairly large distances[2] and are coupled to changes in pore pressure[3–5]. The data suggest to us that a pre-seismic deformation may have occurred around the site of the impending earthquake and that its influence extended as far as the oil wells. The deformation caused changes in pore pressure which gave rise to the large fluctuations in oil flow.

Clearly the evidence presented here is not conclusive. An attempt at detailed explanation of the phenomena has to take into consideration, among other things: (1) that there are some fluctuations in oil flow outside the two periods and (2) that, within the two periods, not all the fluctuations can be correlated with one another. With the present interest in pre-earthquake phenomena, however, we felt that these data should be made known. It would be interesting to see if such phenomena occur in other regions, since they may help in the prediction of earthquakes.

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Alternative interpretation of sulphur isotope ratios in the McArthur lead-zinc-silver deposit

SMITH and Croxford[1] have reported important new measurements of variations among the isotopes of sulphur in the enigmatic stratiform sulphide deposit at McArthur River, Northern Territory, Australia. The data place severe constraints on processes capable of forming the deposit, and Smith and Croxford interpret them to indicate a syn-sedimentary origin with sulphur supplied from two separate sources—one for pyrite and one for galena and sphalerite. We suggest that Smith and Croxford have misinterpreted several important aspects of their data and that they have thereby been led to erroneous conclusions. The isotopic data do not really support their preferred models of mineralisation. Rather, they support the kind of single-sulphur source model demonstrated for many other stratiform base-metal sulphide deposits, in which dissolution of pre-existing pyrite by a circulating, metal-rich but sulphur-poor brine is followed by deposition of base-metal sulphides. Brines of the kind capable of forming such deposits have been reported from the Salton Sea geothermal field[2], from the Cheleken region in Russia[3], in oil fields[4] and in fluid inclusions[5]. The most complete elucidation of such a model is that by Brown[6] for the White Pine deposit in Michigan but the applicability of the model to many deposits has been pointed out by White[7] and others.

Smith and Croxford observed isotopic fractionations between sphalerite and galena ranging from 2.8‰ to 5.7‰. They interpreted the fractionations to mean that isotopic equilibrium was approached, or attained, between an average temperature of 170° C and 220° C. The interpretation is misleading. If, indeed, isotopic equilibrium were attained, the fractionations indicate a temperature range of at least 100° C to 260° C[8]. Equilibrium fractionations in excess of 4‰ have, however, never been achieved under controlled experimental conditions and it has been pointed out by Czamanske and Rye[9] that large and variable fractionations are probably indicative of low temperatures (200° C or less) of formation and isotopic disequilibrium.

Smith and Croxford also reported that $\delta^{34}S_{pyrite}$ increases upward through the stratigraphic section whereas $\delta^{34}S_{galena}$ and $\delta^{34}S_{sphalerite}$ seem to be independent of stratigraphic position. This led them to propose their dual sulphur reservoir model. With respect to pyrite, their observation is typical of, and compelling evidence for, pyrite formed diagenetically in anoxic sediments with sulphur supplied by bacterial reduction of sulphate within a closed system[10]. The failure of $\delta^{34}S_{galena}$ and $\delta^{34}S_{sphalerite}$ to mimic the $\delta^{34}S_{pyrite}$ trend, however, does not necessarily indicate a separate source for sulphur; it may simply reflect a different mode of mineral deposition.

The average value of $\delta^{34}S_{pyrite}$ in the deposit is +6.1‰. Assuming isotopic equilibrium between solution and precipitate, the $\delta^{34}S$ range for H_2S in a brine capable of depositing the McArthur sphalerite and galena is 4‰ to 8‰[5,11]. Although recognising that the data do not indicate isotopic equilibrium, we suggest that the actual isotopic composition of the brine was probably within or close to the suggested range. If, therefore, dissolution of pyrite served to supply sulphur to a circulating brine, the isotopic composition in the resulting fluid would be just about that expected to yield the $\delta^{34}S$ values observed for galena and sphalerite.

If our interpretation of Smith and Croxford's measurements is correct, we would expect to see a systematic relationship between $\delta^{34}S$ in each of the sulphides. In Fig. 1 we have plotted $\delta^{34}S$ for each sulphide against its stratigraphic position (distance above footwall). The distribution of $\delta^{34}S$ of each sulphide about an average line fitted by least squares is striking, and particularly noticeable in the upper and lower parts of the stratigraphic section. We suggest that this striking similarity of local variations of $\delta^{34}S$ means that the sulphides must be genetically related. We therefore propose the following model as the one most nearly accounting for the various observations reported on the McArthur deposit.

The host shales within which the deposits are now found were normal anoxic sediments in which were formed the thin laminae of pyrite, the pyrite framboids and the pyritized sedimentary grains reported by Croxford and Jephcott[12]. The pyrite was formed during diagenesis with sulphur supplied by bacterial reduction of sulphate in a closed reservoir. We suggest that the original upward enrichment of $\delta^{34}S$ was even more pronounced than that now seen in the McArthur deposit—possibly as pronounced as the line BB in Fig. 2. Subsequent to the formation of the pyrite, a base-metal-rich but sulphur-poor brine system was set in motion. A local heat source and nearby evaporite deposit, both helpful but not essential prerequisites for the origin and movement of such a brine, were present at McArthur River[12,13]. The brine was initially undersaturated with respect to pyrite and therefore dissolved some of the pyrite from the shale to produce an isotopically homogeneous solution with a $\delta^{34}\Sigma S$ close to 6‰. As dissolution of pyrite proceeded the brine became saturated with respect to galena and sphalerite, and both minerals commenced precipitating within the shales. Concurrently, other shale-brine reactions (such as those involving silicates and carbonates) were taking place, altering the chemistry of the brine and its solution properties. Eventually a second generation of pyrite was precipitated around the remaining grains of diagenetic pyrite to produce the distinctive and ubiquitous brown-yellow overgrowths reported by Croxford and Jephcott[12,14]. The $\delta^{34}S_{pyrite}$ values reported by Smith and Croxford therefore must be an average of $\delta^{34}S_{diagenetic\ pyrite} + \delta^{34}S_{overgrowth\ pyrite}$. The marked stratigraphic trend of $\delta^{34}S_{pyrite}$ observed by Smith and Croxford, therefore, reflects the effect the sulphur in diagenetic pyrite had on their measurements, and the similarity in distribution between $\delta^{34}S$ for pyrite, galena and sphalerite reflects the influence of the overgrowth pyrite drawing sulphur from the same reservoir as the galena and sphalerite.

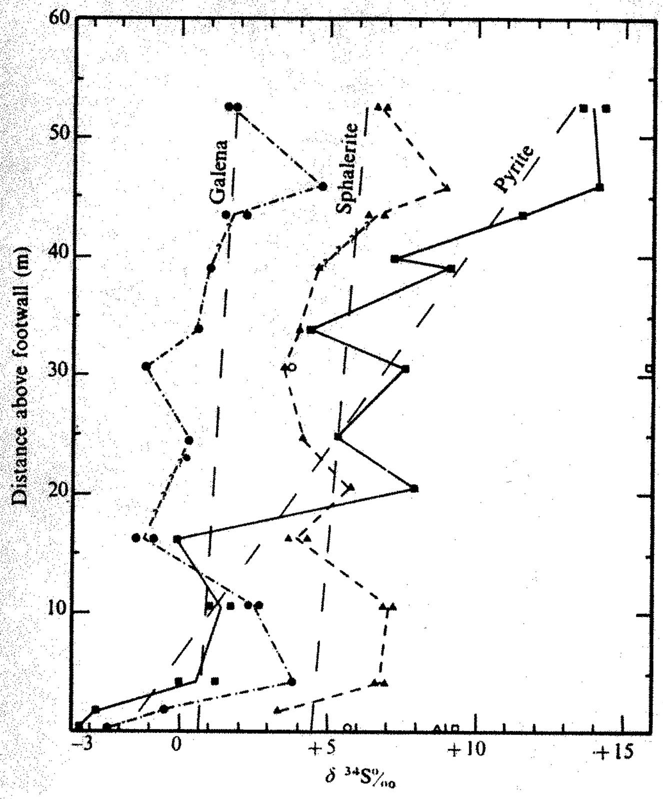

FIG. 1 Variations in the sulphur isotopic composition of galena, sphalerite and pyrite in the McArthur orebody. ●, Galena; ▲, sphalerite; ■, pyrite; — — —, least squares fit line. Solid symbols represent shale mineralisation and open symbols represent slump breccia mineralisation.

An idealised behaviour of sulphur isotopes predicted by our model is presented in Fig. 2. Line AA is the observed stratigraphic trend for $\delta^{34}S_{pyrite}$. Line BB is a postulated $\delta^{34}S$ trend for the original diagenetic pyrite distribution. CC is the $\delta^{34}S$ of an overgrowth pyrite precipitated from the same solution as galena and sphalerite at an average temperature of 170° C[8]. No real significance should be attached to this temperature, because it is not possible to assign an accurate value to the temperature at which the reactions actually occurred. Qualitatively, however, any low temperature will give a similar result, assuming that the $\delta^{34}S$ of pyrite reflects the $\delta^{34}S$ of H_2S in solution. By adding various amounts of overgrowth pyrite to diagenetic pyrite, actual $\delta^{34}S_{pyrite}$ stratigraphic curves can be generated. To illustrate this we have shown two such model curves on Fig. 2, one assuming mixing of 20 weight % of overgrowth pyrite and 80 weight % of diagenetic pyrite (curve DD) and the other assuming mixing of 80 weight % of overgrowth pyrite and 20 weight % of diagenetic pyrite (curve EE). All but two points on the observed $\delta^{34}S$ stratigraphic curve FF lie within the shaded area defined by these model curves, thereby suggesting that our assumed original $\delta^{34}S$ distributions are reasonable and that the actual amount of diagenetic and overgrowth pyrite mixing at McArthur River is between the limits defined by the curves DD and EE. The two points on FF that are not accounted for by our idealised model can be easily accommodated within the shaded field by allowing for small deviations in the original $\delta^{34}S$ values about curve CC.

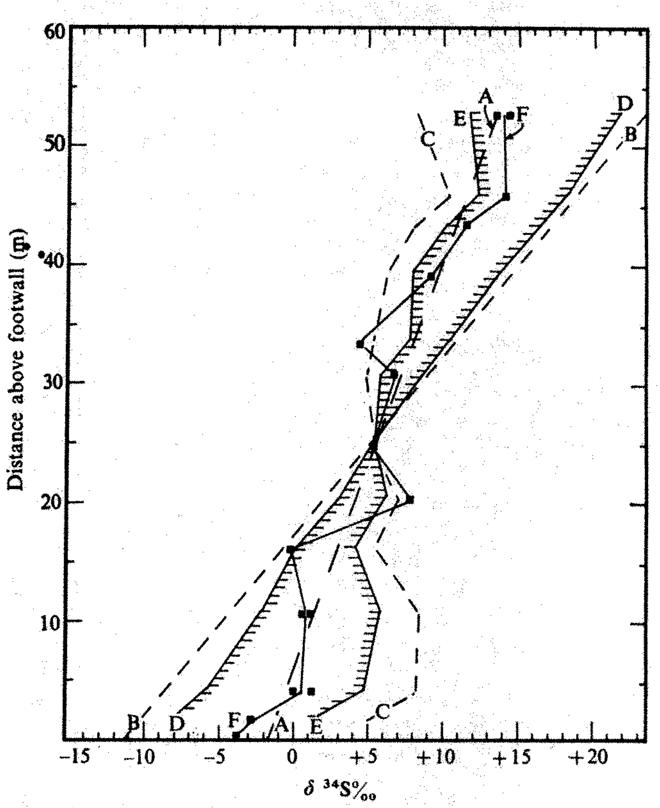

FIG. 2 Variations in the model sulphur isotopic composition of pyrite. AA, Least squares fit line to observed pyrite $\delta^{34}S$ distribution; BB, postulated diagenetic pyrite $\delta^{34}S$ distribution; CC, postulated overgrowth pyrite δ^{34} distribution; DD, predicted model pyrite $\delta^{34}S$ distribution assuming mixing of 20 weight % overgrowth pyrite with 80 wt. per cent diagenetic pyrite; EE, predicted model pyrite $\delta^{34}S$ distribution assuming mixing of 80 weight % overgrowth pyrite with 20 weight % diagenetic pyrite; F——■——F, observed pyrite $\delta^{34}S$ distribution.

Apparently as an afterthought, Smith and Croxford[1] proposed a qualitatively interesting model that turns out to be mathematically identical to our model. In this model they suggest that iron had an exhalative origin and was initially deposited as a monosulphide together with galena and sphalerite. Subsequently, the monosulphide was converted to pyrite by reaction with sulphur provided by bacterial reduction of soluble sulphate. This model can be quantitatively described by Fig. 2. Line CC would then represent the δ^{34}S distribution of the early monosulphide and BB would represent the δ^{34}S distribution of the bacterially produced sulphur. Although mathematically equivalent to our model, this syn-sedimentary model is not texturally equivalent because it cannot satisfactorily explain the pyrite overgrowths present in the McArthur ores[12,14].

We believe that our model offers a possible explanation for other geological features found at McArthur. For example, the predominance of iron-bearing dolomites in the unmineralised sediments stratigraphically above and below the deposit[1] would, in our model, represent remobilised iron that was leached from the shales during the mineralising process. Insufficient data, however, prevent us from commenting on the application of our model to the δ^{34}S ratios from the slump breccia mineralisation.

If the overgrowth pyrite could be separated from diagenetic pyrite it would be possible to test our model. The model predicts that near the footwall sulphur from the overgrowth pyrite should be isotopically heavier than the sulphur from the diagenetic pyrite, whereas near the hangingwall the opposite should be the case.

We suggest, in conclusion, that the sulphur isotopic ratios from the McArthur lead-zinc-silver deposit are better explained by a single-sulphur source, two-stage mineralisation model than by any of the previously suggested dual-sulphur, syn-sedimentary models.

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Structural polytypism of SnS$_2$

ALTHOUGH recent work has indicated that more than one structural type of tin sulphide (SnS$_2$) exists[1], the magnitude of polytypism in this substance has not been studied until now. We have recently grown crystals of tin sulphide and have studied Weissenberg X-ray diffraction photographs of ninety specimens. Here we report the several polytypes which have been observed to date and indicate something about the frequency of the various structures.

The basic structural unit of tin sulphide polytypes consists of two layers of hexagonal close-packed sulphide ions with smaller tin ions nested between them. The arrangement is isotypic with other layered MX_2 compounds, for example, $Mg(OH)_2$, CdI_2, PbI_2, and can be represented in a similar manner by AγB (or BγA, AβC, CβA, BαC or CαB) in which sulphide ions are represented by Roman letters and tin ions by Greek letters in the common notation for the closest packing of spheres. Various stacking arrangements of this unit 'sandwich' layer can produce different polytypes. For example, *type 2H* is $[(A\gamma B)]_n$ and *type 4H* is $[(A\gamma B)(C\alpha B)]_n$. In the polytype name the integer refers to the number of sulphur layers in the unit cell and *H* and *R* indicate hexagonal or rhombohedral symmetry.

Until now *type 2H* has been considered to be the 'normal' SnS$_2$ structure, with $[(A\gamma B)]_n$, $P\bar{3}m1$, $a = 3.6486$ Å, $c = 5.8992$ Å (ref. 2). This structure was determined first by Oftedal[3,4], for synthetic material, and later was recognised as the usual structure of natural tin sulphide (the mineral berndtite) by Moh and others[5–7]. About a quarter of our crystals are *2H*, second only to *18R* in frequency.

Type 4H has been recognised relatively recently. Structural data for this include $[(A\gamma B)(C\alpha B)]_n$, $P6_3mc$, $a = 3.6486$ Å, $c = 11.7984$ Å. On X-ray powder photographs Guenter and Oswald[1] found evidence for the *4H* structure in synthetic materials, and Clark[8,9] detected it in natural berndtite. We have found six crystals of pure *4H* and five other crystals in which *4H* is syntactically coalesced with either *18R* or disordered structures.

Type 18R is the most common structure encountered in our crystals. Out of ninety crystals examined, 38.9% are pure *18R*, while additional *18R* structures are coalesced with other polytypes such as *2H* and *4H*. Disordered structures also usually approach *18R* according to our X-ray films. This polytype was identified earlier using the oscillation X-ray method[10] and its structure is being determined by Inoue, Ishizawa, and Fujiki (in preparation).

Two specimens each of *8H* and *10H* have been encountered in our investigation. Their structures will be reported subsequently. The possibility of a *10H* polytype had been suggested by Karakhanova *et al.*[11] from studies of X-ray diffraction powder patterns but their data were not convincing.

Other crystals, for which there is some uncertainty about the number of layers in the unit structure, are near *14H*, *24H* (or *72R*) and *30H* (or *90R*).

Fourteen of the ninety crystals exhibit more or less random stacking of the layers (disordered structures) normal to the *c* axis. Most of these, however, apparently contain large 'blocks' of the *18R* structure, as indicated by nodes of higher intensity at the *18R* X-ray reflection positions on the continuous streaks on Weissenberg films.

The crystals used in this study were grown by a chemical transport reaction described earlier[12]. The materials used include metallic tin, powdered sulphur, and flake-like iodine. The iodine is used as a transport reagent. The reactions were carried out in vacuum-sealed vycor tubes 13 cm long and 1.1 cm inside diameter. Crystals were grown during transport from the 700° C zone to the 600° C zone. Reaction time ranged from 68 to 142 h. Iodine concentrations varied from 0 to 4 mg cm^{-3}. The crystals are golden-yellow to reddish-brown plates, varying from a few millimeters to 15 mm across. They are tabular parallel to {0001} and usually have an anhedral outline, although hexagonal symmetry can be seen on some of the smaller specimens.

In addition to determining the structures of some of the polytypes mentioned here, we plan to study the relationships between the conditions of growth and the polytypes formed and to consider the possible influence of screw dislocations upon the generation of polytypes. Growth spirals, believed to be related to structural polytypism, have been observed on crystals of the compound[12].

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Filamentous algae from the 2,300 m.y. old Transvaal Dolomite

THE suggestion that stromatolites are analogous with modern algal mats was first made in 1933 (ref. 1). The earliest acceptance of this analogy was for structures in the Transvaal Dolomite[2]. Young described these structures in a series of papers between 1928 and 1943, most of his work being from a spectacular section in the Hol River. This lies 1½ km north of Boetsap, some 50 km north-west of Warrenton in the northern Cape Province (Fig. 1).

Many examples of microfossils are now known from the Precambrian[3]. On present evidence eukaryotic forms extend back in time through the Bitter Springs Formation[4] to the Beck Springs Dolomite of age 1,200 to 1,400 m.y. (ref. 5). Prior to this there are examples of rocks containing structures analogous to filamentous blue-green algae as old as the Gunflint Formation[6], for which an age of $\sim$2,000 m.y. is suggested[7]. Before this there is a gap in evidence of microorganisms back to the Swaziland rocks of the Onverwacht and Fig Tree Groups, which are more than 3,200 m.y. old[8,9]. The Onverwacht contains paired spheroids and filaments of uncertain biologic affinity[10,11], the Fig Tree rod-shaped bacteria[12,13].

The Transvaal Dolomite is older than 1,954 ± 30 m.y., a date for the granites of the Bushveld Complex[14] post-dating the basic phase, the latter being intruded into strata above the Transvaal Dolomite, and younger than 2,300 ± 100 m.y., an age obtained for a single specimen from the underlying Ventersdorp Lavas[15]. Recently Crampton (personal communication) dated the Ongeluk lavas above the Dolomites at 2,224 ± 21 m.y. The unit containing these lavas is, however, separated from the Dolomites by an unconformity of regional extent[16,17] suggesting that this is a minimum age with a figure approaching 2,300 m.y. seeming more likely. The present study fills in part of the gap in the microfossil record between the Gunflint Formation and those of the Fig Tree Group.

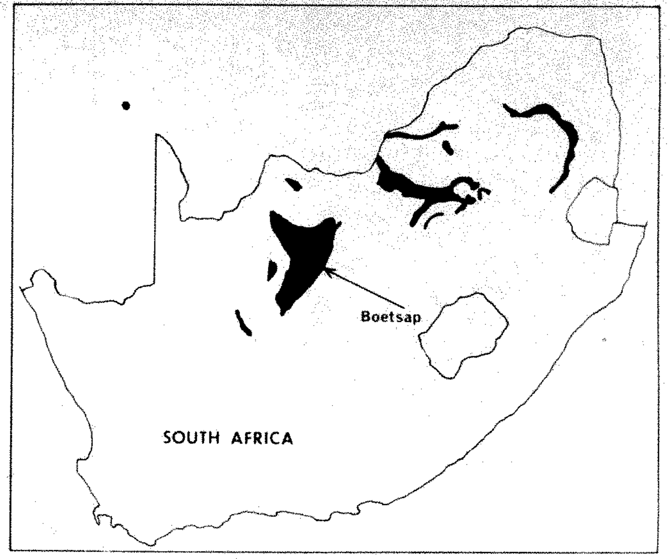

FIG. 1 Locality map, showing the distribution of the Transvaal Dolomite.

Environmental studies in progress in the Transvaal Dolomite have included a re-assessment of Young's Hol River section, now referred to as the Boetsap River section[18]. In this section stromatolitic assemblages dominate an outcrop thickness of no more than 135 m near the base of the Dolomite. A conceptual model has been developed that represents a modification of a limestone shelf model[19], and also an extension of the concept that domes increase in size outward in the intertidal[20,21] beyond this zone into deeper water. It is inferred that accumulation took place through a telescoped range of environments shelving relatively steeply through the intertidal to a high energy agitated zone and into the subtidal. In this model the more striking columnar stromatolites are intertidal, but seaward of the agitated zone more delicate columns may occur within large domes. We note that the assemblage of as yet unstudied, that is unreconstructed, columnar stromatolites from this locality seems

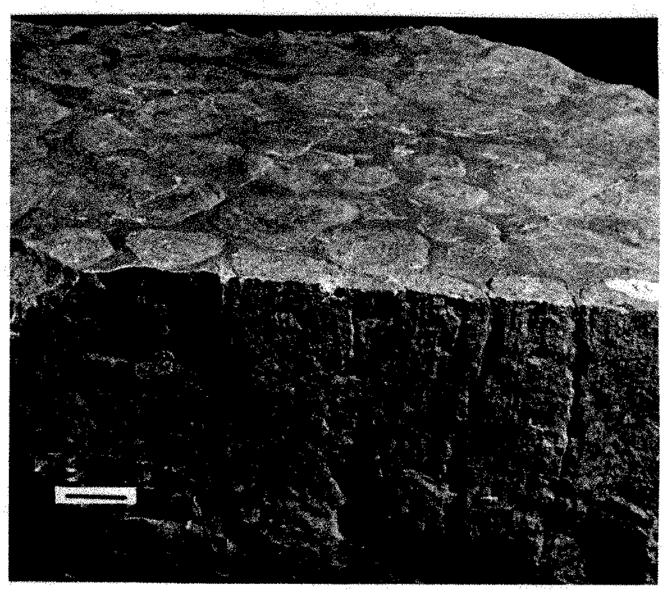

FIG. 2 Intertidal columnar stromatolites. (Scale represents 2 cm).

more diverse than any other pre-Riphean forms described to date. But the analogy of ancient stromatolites and algal mats suggests the presence of microorganisms at the time of formation of any stromatolitic unit, and the purpose of this communication is to document the existence of algal filaments found in association with both subtidal and intertidal columnar forms.

Scanning electron microscopy (by M. MacG.) has revealed the presence of filamentous forms from the Boetsap section. The first of these is recorded from cylindrical columnar stromatolites from the intertidal zone (Fig. 2). The columns are of limestone, the inter-column fill of dolomite. The filaments are narrow, elongate and non-septate. They have a breadth of 1 to 2.5 µm, and a length that may exceed 120 µm (Fig. 3*a*). A looped filament is illustrated in Fig. 3*b*.

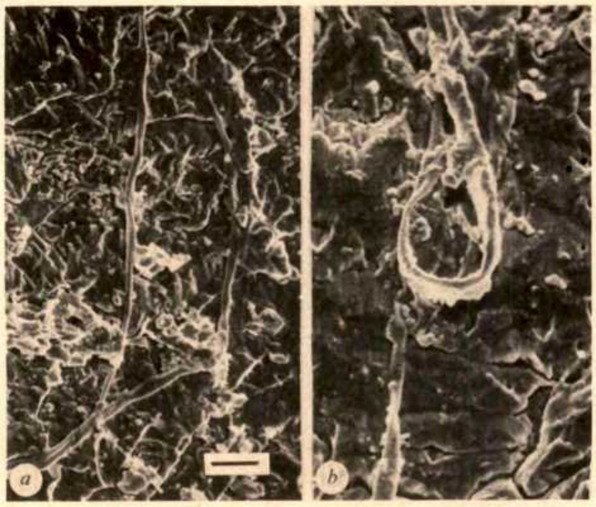

FIG. 3 *a*, Sheathed algal filament. (Scale represents 10 µm). *b*, Looped sheathed algal filament. (scale represents 10 µm).

Two other forms are from small-scale columns (Fig. 4) developed in large elongate subtidal domical stromatolites. The columns are composed of interlayered dolomite and limestone, the inter-column fill of limestone. Figure 5*a* illustrates a slightly curved filament some 70 µm long, tapering at one end from a breadth of 9 µm. Crosswalls are distinct, and the multicellular filament has cells 9 to 14 µm long. The other form is a tapering multicellular filament with an enlarged basal cell (Fig. 5*b*). The basal cell may represent a heterocyst. There are constrictions at the septae, and the filament is curved. It is 160 µm long, and the average breadth is 8 µm. The length-to-breadth ratio of the cells is constant, except for the pointed terminal cell.

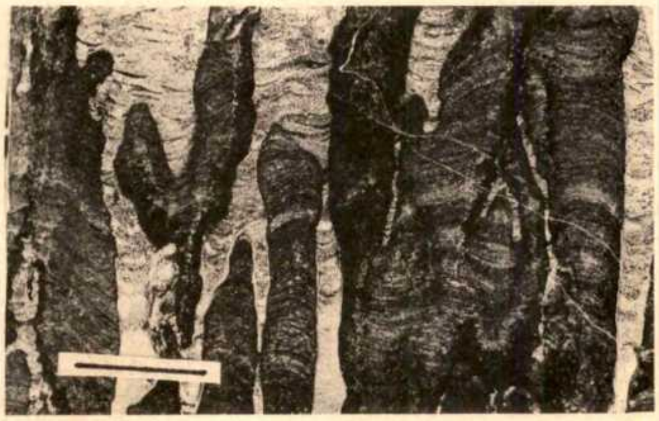

FIG. 4 Subtidal columnar stromatolites. (Scale represents 2 cm).

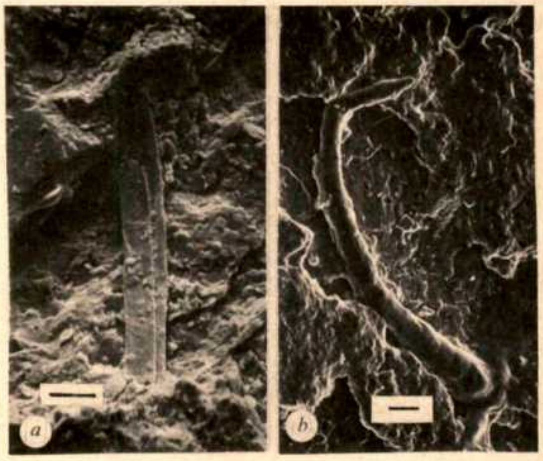

FIG. 5 *a*, Tapering multicellular filament. (Scale represents 10 µm). *b*, Tapering multicellular filament with heterocyst. (Scale represents 10 µm).

The filaments shown in Fig. 3*a* and *b*, although not displaying any cellular structure, share some characteristics with modern sheathed Oscillatoriaceae[22]. The similarity to modern forms of photosynthetic blue-green algae is more distinct for the filaments illustrated in Fig. 5*a* and *b*: the former with a filamentous multicellular form, and no heterocysts or akinetes, to the Oscillatoriaceae; and the multicellular, curved tapering form of the latter, with an enlarged basal cell, to the Rivulariaceae. In this announcement no attempt is made to further characterise these forms, although Fig. 5*b* appears similar to the genus *Rivularia*.

The Oscillatoriaceae and Rivulariaceae are represented in the somewhat younger Gunflint cherts[23], but these Transvaal forms are the oldest blue-green algae from these two families yet recorded. The Transvaal Dolomite is among the oldest extensive shelf carbonates known, that is as distinct from more localised carbonates associated with vulcanicity. If there is a relationship between the accumulation of free oxygen on a large scale and the accelerated precipitation of carbonate sediments[24], then it can be speculated that photosynthetic organisms may not have developed in any great abundance much prior to about 2,300 m.y.

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New route for the quenching of $N_2(A^3\Sigma_u^+)$ in the aurora?

THE volume production rate of $N_2(A^3\Sigma_u^+)$ in the aurora, as estimated from the observed emission rates in the N_2 first positive and second positive systems[1], is an order of magnitude greater than the observed emission rate[2] in the Vergard-Kaplan system[3] ($A^3\Sigma_u^+ - X^1\Sigma_g^+$). This implies a high rate of quenching by other species present in the auroral region. The most prominent species at the appropriate altitudes (>100 km) are N_2, O_2, N, O and NO (ref. 4). Detailed analysis of the quenching rate has led to the prediction that collision with O atoms makes a major contribution to the quenching process. However such quenching in the aurora would have to proceed[1] with an efficiency one or two orders of magnitude greater than observed in the laboratory[5,6] in order to account for the observed V-K intensities.

It has been proposed[7] that one reason for this apparent discrepancy may be errors in the laboratory determination of O atom concentrations. Even so it is clear that our present understanding of the quenching mechanism is incomplete[1,2].

At the University of Kent we have been studying some reactions of gas phase nitrogen atoms, using the nuclear process $^{14}N\ (n, 2n)^{13}N$ (with ~ 14 MeV neutrons produced by a SAMES neutron generator). We have observed that in mixtures of N_2 and O_2, ^{13}N forms only two products, $N^{13}N$ and $N^{13}NO$, which are detected by radiogaschromatography. Fractional yields of $N^{13}NO$ obtained at various oxygen mole fractions are shown in Table 1. It is clear from these results that $N^{13}NO$ is formed readily in the presence of minimal quantities of oxygen.

We believe that these results are consistent with the following elementary reactions:

$$N^{13} + N_2 \rightarrow N^{13}N + N \tag{1}$$

$$^{13}N + N_2 \rightarrow N^{13}N(^3A) + N \tag{2}$$

$$N^{13}N(^3A) + M \rightarrow N^{13}N(^1X) + M \tag{3}$$

$$N^{13}N(^3A) + O_2 \rightarrow N^{13}NO + O \tag{4}$$

The assignment of the $A^3\Sigma_u^+$ state to the electronically excited species proposed for these reactions is consistent with, first, the very slow quenching rate of $A^3\Sigma_u^+$ in N_2 ($k \sim 10^{-20}$ cm³ s⁻¹)[8] and, second the high radiative lifetime of $A^3\Sigma_u^+$, ($\tau \sim 2$ s)[3].

TABLE 1 Fractional yield of $N^{13}NO$ at various O_2 mole fractions in N_2/O_2 mixture.*

O_2 mole fraction	Fractional yield of $N^{13}NO$
0.51	0.48 ± 0.05
0.25	0.48 ± 0.05
0.12	0.42 ± 0.05
$\sim 10^{-5}$†	0.19 ± 0.05‡

* All results refer to N_2/O_2 mixtures at a total pressure of 5 atm in a 20 ml reaction vessel.

† This figure is intended as an order of magnitude indication only. The gas was commercial "oxygen free nitrogen" (BOC Ltd).

‡ This figure represents an average value obtained from several different samples of N_2, obtained from different gas cylinders and irradiated in different reaction vessels.

If this assignment is correct, then reaction (4) becomes an exoergic process, the amount of energy released depending on the product states. If these are N_2O ($X^1\Sigma^+$) and $O(^3P)$, then the energy released is about 70 kcal mol⁻¹. N_2O formed in the aurora by the mechanism proposed above would be expected to disappear fairly rapidly. Indeed mass spectrometer measurements have indicated only small amounts of mass 44 species in the thermosphere[9] (and resolution into CO_2 and N_2O has not yet been confirmed) and only small quantities of N_2O are found in the lower stratosphere[10]. Apart from photochemical destruction, possible chemical reactions which would result in the removal of N_2O are reactions (5) to (7).

$$N(^2D) + N_2O \rightarrow N_2 + NO \tag{5}$$

$$O + N_2O \rightarrow N_2 + O_2 \tag{6a}$$

$$(\Delta H_0 = -78.9\,\text{kcal mol}^{-1}\text{ for }O(^3P))$$

$$\rightarrow 2NO \tag{6b}$$

$$(\Delta H_0 = -35.9\,\text{kcal mol}^{-1}\text{ for }O(^3P))$$

$$NO + N_2O \rightarrow NO_2 + N_2 \tag{7}$$

$$(\Delta H_0 = -32.5\text{ kcal mol}^{-1})$$

These reactions have been studied under laboratory conditions[11-13].

It seems likely that $N(^2D)$ is the precursor of much of the NO formed in the mesosphere and thermosphere[10]. Recent measurements of the rate constants for (5) and (8), at ~ 300°K,

$$N(^2D) + O_2 \rightarrow NO + O \tag{8}$$

indicate that the two processes are of comparable efficiency ($k \sim 4 \times 10^{-12}$ cm³ s⁻¹)[14], which is surprising in view of the large number of highly exothermic adiabatic channels open to reaction (5). Experimental determination[12,13] of the activation energies for reactions (6), involving ground state $O(^3P)$ atoms, (14.5 and > 15.5 kcal mol⁻¹ respectively) and reaction (7) (~ 50 kcal mol⁻¹) suggest that these reactions may be less favourable on energetic grounds, although recent rocket measurements[4] of O and NO indicate considerably greater densities of these species than had previously been contemplated. The prominence of the oxygen ($^1S - {}^1D$) transition in the aurora[15] suggests that one should also consider reactions (6) with $O(^1D)$ atoms, in which instance the reaction efficiency is greatly increased, ($k \sim 2 \times 10^{-10}$ cm³ s⁻¹)[14].

These considerations suggest that N_2O may be one of the species produced in the aurora as a result of the chemical reaction of $N_2(A^3\Sigma_u^+)$ with oxygen. A number of chemical processes, as well as photodissociation, are expected to result in the rapid destruction of any N_2O formed. Nevertheless a measurement of the N_2O density in the aurora would be most welcome.

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BIOLOGICAL SCIENCES

Model for the Secondary Structure of the Denatured Conformer of Yeast $\mathrm{tRNA}_3^{\mathrm{Leu}}$

YEAST $\mathrm{tRNA}_3^{\mathrm{Leu}}$ is one of the more thoroughly investigated tRNA species because it can exist in two conformations which can be interconverted[1–5]. The native (N) conformer has full biochemical activity, whereas the denatured (D) conformer is inactive with respect to aminoacylation. Although other tRNAs can be reversibly denatured[6–8], the D conformer of yeast $\mathrm{tRNA}_3^{\mathrm{Leu}}$ is quite stable[1–5]. Because of these properties, yeast $\mathrm{tRNA}_3^{\mathrm{Leu}}$ has been studied in the hope that it might provide insight into the synthetase recognition problem. This aim has been thwarted, however, by lack of information on the secondary structure of the D conformer. Even less is known about the tertiary structure of this molecule in solution.

Fresco noted that both the DHU and TψC loops have regions complementary with the bases in the anticodon loop, and suggested that in the D conformer the anticodon loop is hydrogen-bonded, possibly to a complementary sequence in the TψC loop[6]. This possibility, and disruption of the DHU stem, are the only two structural features which were specified for the D conformer. The presence or absence of the other stems from the clover-leaf structure was not discussed.

High resolution nuclear magnetic resonance (NMR) spectroscopy has made possible the determination of the secondary structure[9–16], and in favourable cases the tertiary structure of tRNA molecules in solution (ref. 15 and unpublished results of D. R. K., Y. P. W. and S. H. C.). We have already examined the high resolution NMR spectra of the N and D conformers of yeast $\mathrm{tRNA}_3^{\mathrm{Leu}}$ at 220 MHz[16]. Two developments prompted us to investigate this problem further: there has been substantial progress in the interpretation of the low field NMR spectra of tRNA molecules, and the 300 MHz spectrometer now provides much improved spectra.

Pure $\mathrm{tRNA}_3^{\mathrm{Leu}}$ was prepared as reported recently[17,18]. A solution of yeast tRNA was heated at 60° C for 5 min in the presence of 10 mM $\mathrm{MgCl_2}$ and cooled to 25° C. This 'heat activated' tRNA was subjected to chromatography in two consecutive columns of BD-cellulose, in the presence and absence of magnesium ion. The partially purified $\mathrm{tRNA}_3^{\mathrm{Leu}}$ was converted specifically into its denatured form ($\mathrm{tRNA}_3^{\mathrm{Leu}}$D) and purified in a column of Sephadex G-100. Based on ^{14}C-leucine acceptance and $\mathrm{T_1}$ RNase degradation, it was judged to be more than 90% pure. The tRNA in cacodylate buffer (10^{-2} M, *p*H 7) was denatured by incubation with EDTA (10^{-2} M) at 60° C for 5 min. The solution was then concentrated by vacuum dialysis against the cacodylate buffer to 60 mg tRNA $\mathrm{ml^{-1}}$.

Spectra were obtained with a Varian Associates HR 300 NMR spectrometer operating at 300 MHz and were time averaged to improve signal-to-noise. Sample volumes were ~0.12 ml and the temperature was controlled to ±1° C. To determine the number of exchangeable protons per tRNA molecule in the low field region of the NMR spectra the integrated intensity in the appropriate spectral region of the $\mathrm{tRNA}_3^{\mathrm{Leu}}$ sample (11–15 p.p.m.) was compared with the integrated intensity of a standard sample of yeast $\mathrm{tRNA}^{\mathrm{Phe}}$

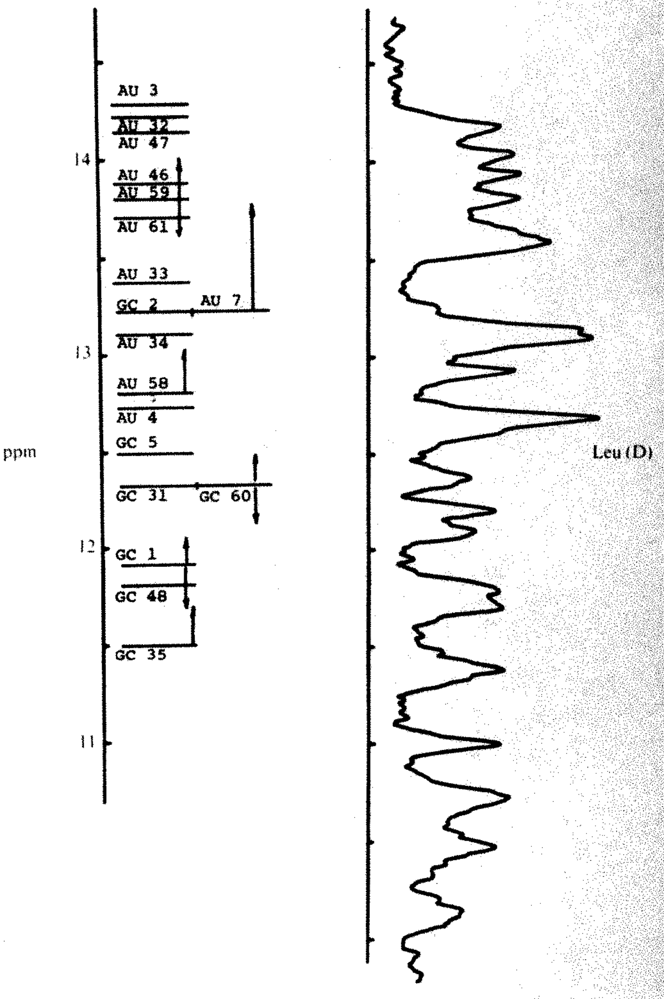

Fig. 1 The 300 MHz proton NMR spectrum of the denatured conformer of yeast $\mathrm{tRNA}_3^{\mathrm{Leu}}$ at 45° C in a solution containing 0.1 M NaCl and 10 mM cacodylate buffer at *p*H 7. The tRNA concentration was 60 mg $\mathrm{ml^{-1}}$. A stick diagram representation of the NMR spectrum computed for the model shown in Fig. 3 is also presented, together with assignments. The length of one of the smaller horizontal bars represents one resonance.

(1.4 mM) which exhibits 19 resonances per tRNA in the 11 to 15 p.p.m. region[12,13]. The accuracy of this comparison is good to ±10%. Alternatively, we assumed that four peaks located between 14.2 and 13.7 p.p.m. in the NMR spectrum of tRNA$_3^{\mathrm{Leu}}$D corresponded to four protons per molecule and then used this to integrate the rest of the spectrum. Both methods gave a value of 18±2 resonances per molecule.

The low field proton NMR spectrum of the denatured form of tRNA$_3^{\mathrm{Leu}}$D is shown in Fig. 1. In previous studies we demonstrated that the hydrogen-bonded ring NH protons of Watson-Crick base pairs (AU, Aψ or GC) give rise to one resonance per base pair in the low field (11 to 15 p.p.m.) region of the NMR spectrum[9–15]. Therefore, integration of the spectrum by the two methods described above indicates that the D conformer contains 18±2 base pairs, and an accurate comparison of the N and D conformer spectra indicates that the D conformer contains ~4 less resonances than the N conformer[16]. This comparison further indicates that the difference between the N and D conformers cannot be explained in terms of a loss of three base pairs. This is more clearly emphasised by the difference spectrum (Fig. 2) obtained by subtracting the spectrum of the N conformer from that for the D conformer. This suggests that in the change from the N to the D conformer at least five of the original base pairs are lost and at least two base pairs are gained. Since the spectrum of the native conformer can be accounted for accurately in terms of the standard clover-leaf structure (unpublished results of D. R. K., Y. P. W. and S. H. C.) the denatured conformer cannot have a clover-leaf structure. To deduce the probable secondary structure of the D conformer we used a set of semi-empirical rules we have developed for relating the secondary structure of a polynucleotide to the exact location of the low field resonances[12–14]. Resonances from AU and GC base pairs in a double helix are upfield shifted from their intrinsic (unshifted) positions as a result of ring current fields generated by neighbouring bases, and Table 1 lists a set of parameters which give a satisfactory account of the NMR spectra of other tRNA species, tRNA fragments and model systems. These shift parameters when combined with the unshifted positions (AU°=14.5 p.p.m., GC°=13.6 p.p.m. and Aψ°=13.5 p.p.m.) enable us to compare the low field NMR spectrum expected for any helical structure[14,15].

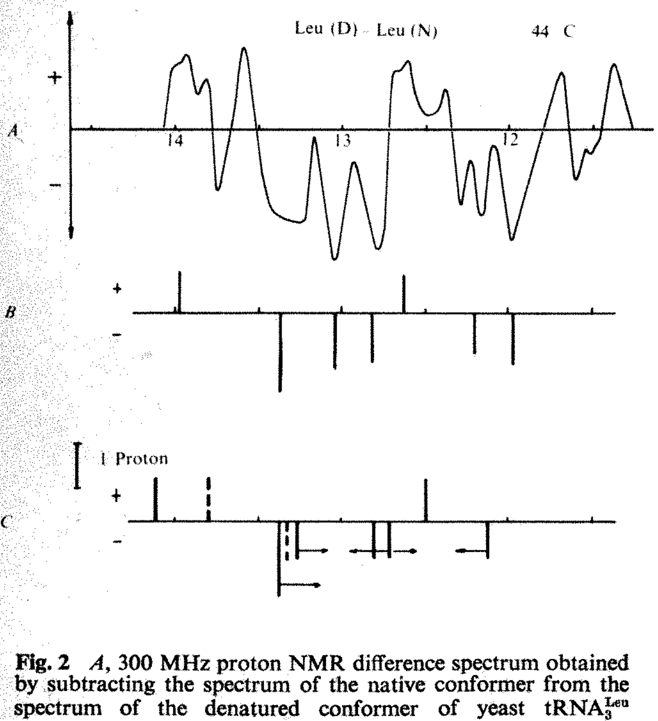

Fig. 2 *A*, 300 MHz proton NMR difference spectrum obtained by subtracting the spectrum of the native conformer from the spectrum of the denatured conformer of yeast tRNA$_3^{\mathrm{Leu}}$ *B*, A stick diagram representation of the observed D–N difference spectrum. *C*, The computed D–N difference spectrum based on the assumption that the N conformer has the cloverleaf structure and the D conformer has the structure shown in Fig. 3. Some terminal base pairs may be susceptible to end effects and the expected range for these resonances is also indicated. If the amino acid acceptor stem and the TψC stem are not stacked in the D conformer, there will be additional changes (indicated by dashed lines).

Table 1 Summary of ring current shift parameters used in calculation of low field proton NMR spectra of tRNA

5′	3′	5′	3′
U =0 C =0 G =0 A =0.1	A =1.1 G =0.6 C =0.1 U =0	U =0 C =0 G =0 A =0	A =1.1 G =0.7 C =0.2 U =0.1
UA ∩∀			CG Ͻƃ
U =0.1 C =0.2 G =0.6 A =0.6	A =0 G =0 C =0 U =0	U =0.1 C=0.25 G =0.7 A =1.0	A =0 G =0 C =0 U =0
3′	5′	3′	5′

The notation 5′ and 3′ refers to the sugar positions at the ends of chains containing the neighbouring bases indicated. The unshifted positions for usual base pairs are: (AU)°=14.5 p.p.m., (GC)°=13.6 p.p.m. and (Aψ)°=13.5 p.p.m.

Since the D conformer competitively inhibits amino-acylation to the N conformer[4], some features of the original clover-leaf structure probably remain, and for various reasons we assume that the amino acid acceptor stem is retained (unpublished results of D. R. K., Y. P. W. and S. H. C.). We then examined various models containing one of the following additional structural features: (1) TψC stem+anticodon stem; (2) TψC stem+minor stem; (3) anticodon stem+minor stem. Using the shift parameters of Table 1, NMR spectra were computed for all possible base pairing schemes consistent with these restrictions. The only one which gave a satisfactory fit of the low field spectrum of the D conformer and, perhaps more importantly, the native-denatured difference spectrum, is shown in Fig. 3. The NMR spectrum predicted for this model is shown in Fig. 1 and the calculated (N-D) difference spectrum is compared with the observed difference spectrum in Fig. 2.

The positions of resonances associated with some of the terminal base pairs are sensitive to the stacking of adjacent bases and the range of values expected are also indicated in both Figs 1 and 2 by arrows which show the possible range of shifts. The resonances from AU$_7$ and AU$_{58}$ are particularly sensitive to the stacking of the amino acid acceptor stem and the TψC stem, and arrows in Fig. 2 indicate how these two resonances would shift if the two arms were unstacked. Our earlier work with yeast tRNA$^{\mathrm{Phe}}$ suggests that AU$_7$ could be absent from the low field spectrum because of the adjacent GU mismatch[12,13]. If this is true, we cannot draw any conclusion regarding the relative orientations of the amino acid acceptor stem and the TψC stem. If the AU$_7$ resonance is present, however, the best fit is obtained when these two stems are stacked.

The model we propose for the D conformer differs from the clover-leaf in that both the DHU stem and the anticodon stem are replaced by a single 'extra' helix which involves pairing between bases of the anticodon loop and the TψC loop in the clover-leaf model. Conversion from the D to the N conformer necessarily involves removing the 'extra' helix and this could account for the large ΔH which is observed experimentally[6].

The only other data bearing directly on the base pairing structure of the D conformer are the oligonucleotide binding data of Uhlenbeck *et al.*[5]. These experiments show that in the D conformer, the DHU loop and stem regions are quite accessible to base pairing with complementary oligonucleo-

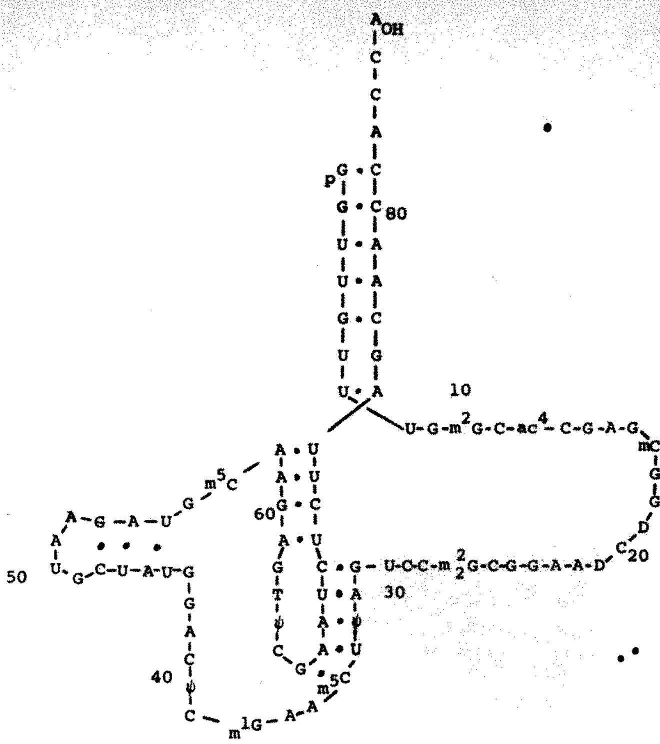

Fig. 3 Proposed model for the secondary structure of the denatured conformer of yeast $tRNA_3^{Leu}$.

tides whereas the anticodon loop region which is exposed in the N conformer is 'protected' in the D conformer. These observations are also consistent with the proposed structure. A more complete account in which the results for alternative structures are presented along with more extensive data on both the native and denatured conformers is in preparation.

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Purification of Bacteriophage λ Int Protein

BACTERIOPHAGE λ can direct the formation of small amounts of several λ proteins in a host whose endogenous protein synthesis has been decreased by ultraviolet irradiation before phage infection[1]. The phage protein can be labelled by the incorporation of radioactive amino acids after infection. Many of these proteins can be resolved by sodium dodecyl sulphate (SDS) gel electrophoresis and tentative gene identifications can be made by comparing the products of wild type phage infection with those of phage carrying mutation(s) which selectively remove certain gene product(s)[2]. I have purified the *int* gene product using differential label techniques to detect the Int polypeptide. This gene product functions *in vivo* to direct recombination between specific sites in DNA; the radiochemically pure product has been used to investigate the *in vitro* interaction between Int and DNA.

Figure 1 presents a typical double label experiment which confirms the finding[2,3] of the production of a polypeptide of molecular weight 42,000 dependent on a functional *int* gene. Only this polypeptide is made in significant excess when int^+ phage products are compared with those from an unsuppressed *int* amber (*int* 29) phage. The absence of this polypeptide in *int* 29-infected cells probably does not reflect a polar effect of the amber mutation in the *int* gene on another gene since polypeptide production in this system involves gene N function[5] and polarity effects of amber mutations are not seen during transcription under the influence of gene N product (personal communication from S. Adhya, M. E. Gottesman and B. de Crombugghe, in preparation). That this polypeptide represents the product of the *int* gene and not the product of another gene under *int* gene control can be deduced from the data in Table 1. In this experiment the production of a polypeptide of molecular weight 42,000 in excess of that produced by phage carrying an *int* nonsense mutation is examined for phage carrying either an int^+ gene or one of five different non-suppressible *int* mutations. Four of the five mutant phage yield amounts of this material experimentally indistinguishable from that of wild type, indicating that production of the polypeptide does not depend indirectly on *int* gene function and therefore the polypeptide represents the product of the *int* gene. *int* 41 may represent a promoter mutation, a nonsense mutation responding to a suppressor not tried (*su* 1–5 have been examined), a small deletion or deletion-substitution and so on.

The appearance of differential label of an appropriate mobility on SDS gel electrophoresis can be used as an assay for Int protein. Using this approach I have devised a purification scheme that reproducibly yields a 75% or greater radiochemically pure Int protein. The purification procedure, based on that for T7 endonuclease described by Center and Richardson[7], avoids the use of detergents or other known denaturants.

Dissociation of protein into individual polypeptide chains is used only in the SDS gel assay. Figure 2*a* shows the elution profile of radioactivity for the final column step and Fig. 2*b* shows a typical SDS gel profile of the purest material. 79% of the ^{3}H-counts recovered from this gel are in a single band corresponding to molecular weight 42,000. In a typical purification procedure, about 10% of the Int detected after the sonication step is recovered from the phosphocellulose column. The molar amount of Int protein can be calculated from its radiochemical activity using an assumed specific activity for leucine in Int (equal to that of the labelling medium) and an assumed leucine content for Int (35 mol leucine mol^{-1} Int protein). Such a calculation indicates the formation of about 50 ng Int product per 200 ml of infected ultraviolet-irradiated cells. At least twenty times more Int can be recovered by applying the identical purification scheme to unirradiated cells which have produced Int after thermal induction of a λ prophage (data not shown).

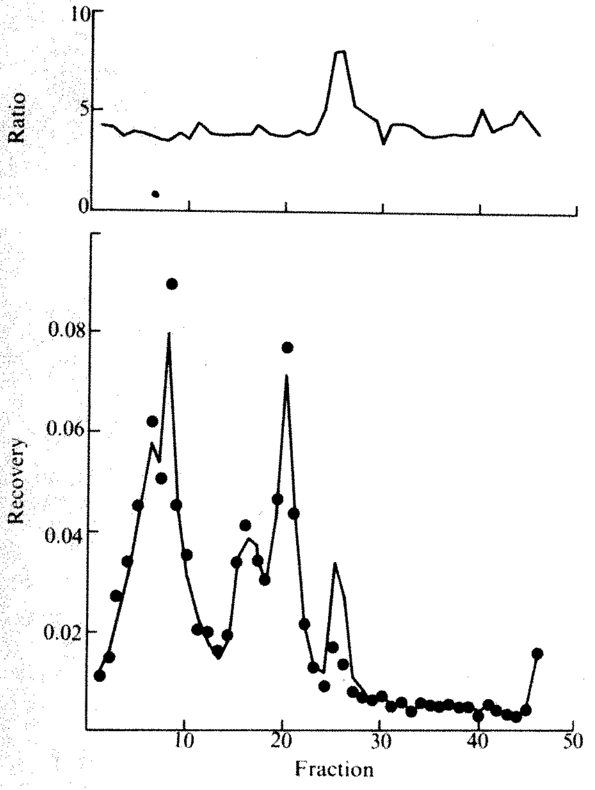

Fig. 1 SDS gel electrophoresis of protein made in ultraviolet-irradiated hosts. *E. coli* strain M159 UV^s, *su*$^-$ was grown on 400 ml medium A with 0.4% maltose at 38° C. At mid-log phase the cells were chilled and irradiated with ultraviolet light (8 min at 25 cm from a 15 W GE germicidal lamp). $MgSO_4$ was added to 0.02 M, the culture was divided and each half was infected with a multiplicity of infection=3 of λ*b*2 *c*1857 or λ*b*2 *int* 29 *c*1857. After 5 min adsorption at 38° C, 2.0 mCi 4-5-^{3}H-leucine (2.6 Ci mmol^{-1}) or 0.2 mCi U-^{14}C-leucine (261 mCi mmol^{-1}) were added to each half. After 60 min of slow shaking at 38° C the cells were chilled, spun, washed twice in 0.02 M Tris-HCl, *p*H 7.4, mixed with a ten-fold excess of untreated cells in the same buffer, disrupted by sonication (four bursts of 5 min with 5 min between bursts, temperature $<10°$ C), and spun at 10,000*g* for 15 min. A sample was made 1% in SDS, 1% β-mercaptoethanol, and 6% sucrose and run in a 7% polyacrylamide gel with SDS according to the procedure of Weber and Osborn[4]. A 7.5 cm gel was cut into forty-six equal fractions, dissolved overnight in 0.5 ml 30% H_2O_2 at 50° C, suspended in a scintillation cocktail containing toluene, 0.85% Beckman TLA, and 16.6% Beckman Biosolv BBS3, and counted. The ratio of c.p.m. in each fraction to total c.p.m. for all fractions is plotted on the lower ordinate as a continuous line for ^{3}H and as discrete points for ^{14}C. The ratio of ^{3}H c.p.m. to ^{14}C c.p.m. for each fraction is plotted on the upper ordinate. 13,530 c.p.m. and 3,300 c.p.m. of ^{3}H and ^{14}C respectively, were recovered from the gel. Direction of electrophoresis is from left to right. Standards of chymotrypsinogen, ovalbumin, catalase and β-galactosidase were run in parallel gels for molecular weight calibration. These proteins (of molecular weight 26,000, 43,000, 60,000 and 130,000) migrate to positions corresponding to fractions 13, 20, 27, 33 and 40, respectively.

Table 1 Int Protein Production by Int Mutants in Ultraviolet-irradiated Cells

Phage	% Incorporation into Int *
λ*b*2*int*$^+$ *c*1857	3.98
λ*b*2*int*21 *c*1857	3.83
λ*b*2*int*44 *c*1857	3.39
λ*b*2*int*4 *c*1857	3.33
λ*b*2*int*6 *c*1857	2.12
λ*b*2*int*41 *c*1857	0.01

The proteins made after infection by each phage listed were labelled with ^{3}H-leucine. In each case, a control culture, infected with λ*b*2*int*29 *c*1857, was labelled with ^{14}C-leucine. Growth of M159, irradiation, infection, labelling, sonication and electrophoresis were as in Fig. 1. The *int* mutations used were isolated by Gottesman and Yarmolinsky[6].

* % Incorporation into Int is ((^{3}H c.p.m. at molecular weight 42,000/total ^{3}H c.p.m. recovered from the gel)−(^{14}C c.p.m. at molecular weight 42,000/total ^{14}C c.p.m. recovered from the gel)) ×100.

Although the material of Fig. 2*b* is nearly homogeneous in radioactive label, it contains greater than 1,000-fold excess of unlabelled protein, none of which is Int protein (or any other λ protein.) Nevertheless, the sedimentation behaviour of this crude Int protein in the presence and absence of its putative DNA substrate can be studied by observing the radiochemical label.

By comparison with marker proteins of known sedimentation coefficient, Int has $S_{20,w}=3.75$ in sucrose gradients containing 0.05 or 0.2 M potassium phosphate buffer. The sedimentation coefficient is characteristic of globular proteins of molecular weight about 50,000.

If Int is mixed with λ DNA before layering on the gradient, the sedimentation behaviour changes markedly. Most of the Int protein sediments with the DNA at $S_{20,w}=30$ with a smaller amount of Int protein sedimenting near the top of the gradient at the 3–4S value seen for Int alone. The binding of Int to DNA becomes progressively weaker as the ionic strength increases and is undetectable in gradients containing 0.3 M potassium phosphate, indicating an electrostatic component of the binding reaction. Under all conditions, the binding is destroyed by prior heating of Int to 65° C for 15 min. For a given amount of Int protein, the proportion of Int bound to DNA increases with increasing amounts of DNA added to the incubation mixture. An indication of the strength of binding can be obtained from the molar concentrations of Int protein and DNA that yield half maximal binding. In 0.05 M potassium phosphate buffer, half binding of 1×10^{-10} mol l^{-1} Int is seen at 1×10^{-10} mol l^{-1} DNA. This apparently tight binding has been examined using DNAs with different attachment site composition in an attempt to discover a component of binding specific for the putative substrate of Int. Over a wide range of ionic strengths, DNA concentrations, divalent cation, ATP and SAM concentrations, no significant difference was seen for Int binding to phage DNA with 0, 1 or 2 attachment sites per genome (λ*b*538, λ, and λ *att*^{2}, respectively[8,9]). Indeed, Int seems to bind as well to phage T4 and phage T3 as well as that of phage λ, indicating that the binding is not specific for a nucleotide sequence unique to λ nor for a pattern of methylated bases.

The tight but non-specific binding of Int to DNA must be reconciled with the highly specific recombination event which is sponsored by Int. Several possibilities are that: (*a*) Int is not the site-specific element in integration and does not show

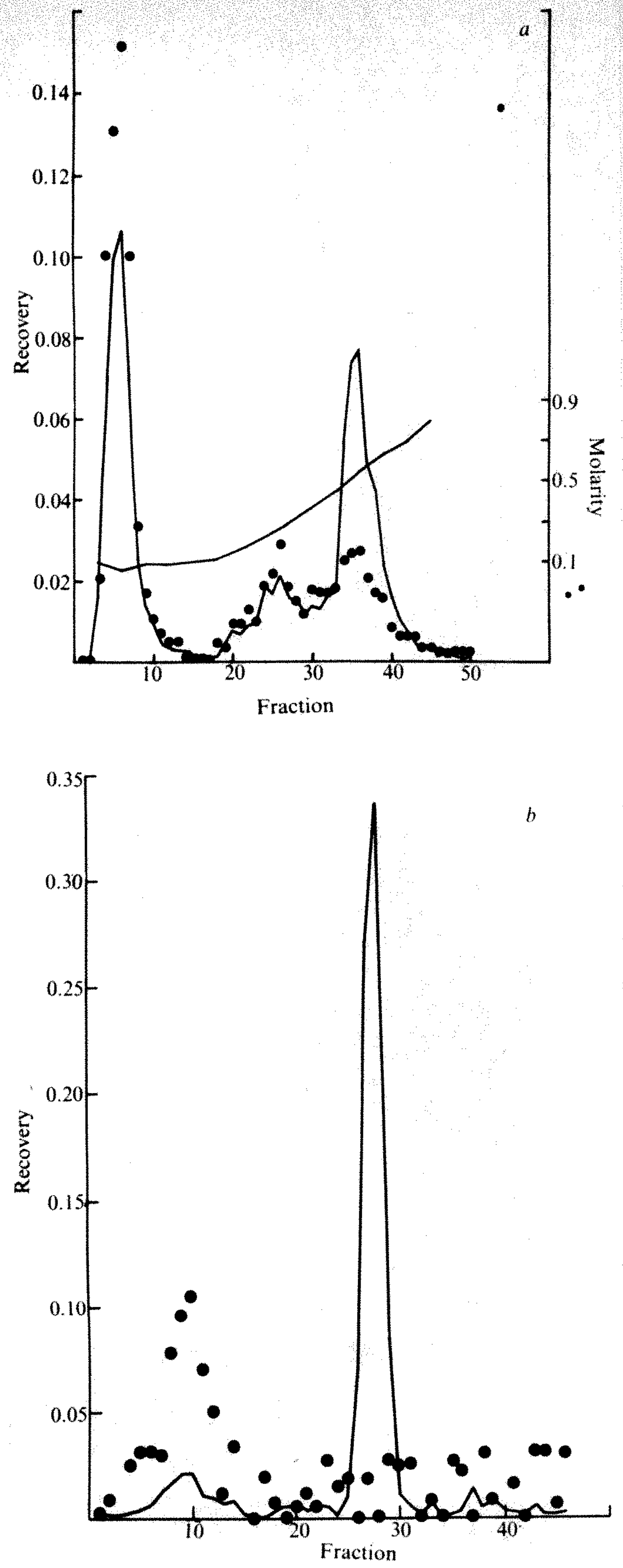

Fig. 2 *a*, Phosphocellulose column elution profile of partially purified radiochemically labelled Int protein. To 147 ml of the sonic extract described in Fig. 1, 24.5 ml of 5% streptomycin sulphate was added. After stirring, the mixture was spun and the pellet resuspended in 23.5 ml 0.05 Tris-HCl, *p*H 8. After this, all solutions were 0.01 M in β-mercaptoethanol unless otherwise stated. The resuspended streptomycin pellet was fractionated with ammonium sulphate; the material precipitating between 40 and 70% saturation was resuspended in 2 ml 0.02 M potassium phosphate buffer, *p*H 7.5, and dialysed overnight against 0.01 M potassium phosphate buffer, *p*H 7.5. A 3 ml sample of the dialysed material was loaded onto a 40 ml column of Whatman DE52 equilibrated with the same buffer and was immediately eluted with 0.2 M potassium phosphate buffer, *p*H 7.4. A fraction representing the peak of total protein, with an $A_{280/260}$ ratio greater than 0.75 was loaded onto a 3 ml column of Whatman P11 phosphocellulose equilibrated with 0.1 M potassium phosphate buffer, *p*H 7.4. The column was washed with one bed volume of this buffer and a linear gradient of potassium phosphate buffer, *p*H 7.4, was used for elution. Samples (1 ml) were collected and aliquots were taken for radioactivity and conductivity. The ratio of c.p.m. in each fraction to total c.p.m. for all fractions is plotted on the left ordinate as a continuous line for ^{3}H and as discrete points for ^{14}C. Molarity of phosphate buffer in each fraction is plotted on the right ordinate. *b*, SDS gel electrophoresis of Int protein purified from ultraviolet-irradiated cells. Fractions 34–38 of the phosphocellulose column elution were pooled. A sample was made 1% SDS, 1% β-mercaptoethanol, heated to 55° C for 30 min, then dialysed against 0.01 M β-mercaptoethanol, 0.1% SDS and 0.01 M sodium phosphate buffer, *p*H 7.4, overnight. SDS gel electrophoresis and subsequent analysis were performed as in Fig. 1. 948 c.p.m. and 76 c.p.m. of ^{3}H and ^{14}C were recovered from the gel.

specific interaction with attachment sites *in vivo* or *in vitro*, (*b*) the specific binding of Int has been lost during purification either through denaturation or removal of accessory proteins or factors, or (*c*) Int, as purified, does possess specific binding, but this is masked experimentally by a large non-specific component. With regard to the latter possibility, the binding of Int to DNA may be similar to the binding pattern of RNA polymerase to T7 DNA and of cyclic AMP receptor protein to *gal* or *lac* DNA. In both of these cases, highly purified, highly active protein preparations which exhibit specific action on certain DNA sites *in vivo* and *in vitro* show tight but predominantly non-specific binding to DNA. Specific binding of the receptor protein to catabolite-sensitive sites has not yet been discerned[10]; in the case of RNA polymerase, binding which reflects the *in vivo* specificity for promoter sites has been revealed after an extensive search for the binding variables[11].

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Acid Protease Activity in *Plasmodium falciparum* and *P. knowlesi* and Ghosts of Their Respective Host Red Cells

ERYTHROCYTIC stages of the malarial parasite apparently obtain the bulk of their amino acids from digestion of host cell haemoglobin[1-5]. In addition, intracellular degradation of unnecessary organelles occurs within the parasite following invasion of the host cell. Thus, it is rather surprising that there have been relatively few studies on the proteolytic enzymes of *Plasmodium*, since these would presumably be involved in both processes. Cell-free extracts of *P. gallinaceum* degraded denatured globin at *p*H 6.5, while haemoglobin was digested very slowly[6]. Data of Cook *et al.*[7] indicate the existence of two proteinases, with *p*H optima of 4 and 8 (*P. berghei*) and 5 and 8 (*P. knowlesi*). In each case, the alkaline protease was the more active.

We have found that the specific activity of an acid protease in *P. berghei*-infected red cells is five to ten times that of uninfected cells[8]. The enzyme degrades haemoglobin optimally at *p*H 2.5 to 3, and there is little activity in the alkaline range. Its properties are very similar to those of a protease from host cell ghosts.

In this report we describe some properties of proteases from *P. falciparum*, *P. knowlesi*, and the red cell ghosts of their corresponding hosts, Aotus and Rhesus monkeys, respectively. Our data indicate: (1) each species of parasite contains a protease that degrades haemoglobin optimally at *p*H 3.6, and which differs in *p*H response and sensitivity to inhibitors from that of host cell ghosts. (2) The enzymes from the two species of monkeys differ from each other in sensitivity to several inhibitors and in *p*H response. (3) The acid proteases from both ghosts and parasites are extremely sensitive to pepstatin, an inhibitor of proteases such as cathepsin D and are also very sensitive to chymostatin, an inhibitor of chymotrypsin-like proteases.

Most procedures used for obtaining parasites give preparations that are somewhat contaminated with membranes and other materials of the host cell[9-10]. Since host cell proteases are tightly bound to the red cell stroma[11], the parasites could be contaminated with varying amounts of host cell enzyme. We therefore decided to initially study the distribution of proteinase activity between the ghost and parasite-rich fraction, and then to compare the properties of the enzymes from each source.

Red cells were freed of leukocytes by a modification of the procedure of Martin[12], as described elsewhere[8]. In most preparations, well over 90% of the leukocytes were removed. Red cells were then haemolysed in a solution of 0.01 M potassium phosphate, *p*H 6.8, containing 2×10^{-5} M EDTA (PE). The sample was centrifuged for 5 min at 17,500*g*. In control cells, the haemolysate was decanted from the ghosts. The latter were suspended in PE, and both fractions were recentrifuged. The ghosts were washed at least twice in this manner, with the washing fluids being added to the haemolysate. Samples were kept frozen until use.

In infected cells, the haemolysate was decanted from the ghosts and parasites. The ghosts, which formed a clear pellet over the parasites, were separated from the latter. The ghosts and parasites were suspended in PE, all three fractions were recentrifuged. A small amount of ghost material was recovered from the haemolysate and from the parasites. This was pooled

Table 1 Specific Activity and Intracellular Distribution of Acid Proteinase and Acid Phosphate in Normal and Infected Red Cells of Monkeys

	P. knowlesi in Rhesus monkey			*P. falciparum* in Aotus monkey			
	Controls	Infected sample 1	Infected sample 2	Controls	Infected sample 1	Infected sample 2	Experiment 2
Specific activity							
Proteinase, total	8.9 (13)	24.4 (28)	27.6 (33)	7.8 (5.5)	10 (6.7)	10 (12)	14.6 (16)
Haemolysate	6.4	11	11	2.9	3.2	5.4	3.4
Ghosts	86.5	180	180	126	70	61	105
Parasites	—	2,600	2,400	—	630	570	1,150
Acid phosphatase, total	0.583	0.438	0.44	0.15	0.30	0.27	—
Haemolysate	0.609	0.24	0.31	0.17	0.30	0.23	—
Ghosts	1.50	0.41	0.49	0.20	0.25	0.36	—
Parasites	—	2.4	1.1	—	1.8	0.48	—
Distribution							
Proteinase, Haemolysate	42	34	32	50	45	43	15
Ghosts	58	33	29	50	29	11	15
Parasites	—	33	39	—	25	45	71
Acid phosphatase, Haemolysate	81	87.5	90	97	96	95	—
Ghosts	19	9.0	8	3	2.3	3.4	—
Parasites	—	3.5	2	—	1.6	1.3	—
Protein, Haemolysate	91	88.4	94.0	97.8	96.7	97.0	96.9
Ghosts	9	11.2	5.4	2.2	2.3	2.8	2.2
Parasites	—	0.35	0.5	—	1.0	0.27	0.95

P. knowlesi in Rhesus monkeys and *P. falciparum* in Aotus monkeys were maintained as described previously[5]. Rhesus blood was
collected when the parasites were primarily in the ring (40%) and early trophozoite (40%) forms (sample 1) or in the early trophozoite (42%)
and late trophozoite (42%) forms (sample 2). The parasitaemia was 30% in sample 1 and had fallen to 16% by sample 2. Blood from Aotus
monkeys was removed from the animal when 92% of the parasites were in the ring form (sample 1) and the parasitised red cells were main-
tained in an *in vitro* culture system[5,13]. Sample 2 was taken when 66% of the parasites were in the trophozoite stage. The initial parasitaemia
was 37%. In experiment two (last column), blood was taken when the parasites were in the ring (44%) and trophozoite (40%) stages (para-
sitaemia=40%). Proteinase activity was tested at *p*H 3.0, as described elsewhere[8], using haemoglobin as substrate. Acid denaturation of
haemoglobin did not increase the rate of breakdown. Results are expressed as µg of bovine serum albumin equivalents released per h per mg
protein. Acid phosphatase was measured at *p*H 4.5 by the method of Torriani[14], using *p*-nitrophenylphosphate as substrate. Data are expressed
as µmoles *p*-nitrophenol released per h per mg protein. Protein was determined by the method of Lowry *et al.*[15]. Distributions of enzymes
and protein are given as the percentage of the total recovered that was found in a given fraction. Values in parentheses denote the specific
activity as calculated from the sum of the various fractions. The percentage of reticulocytes in the blood of Rhesus monkeys was 0.9%. In
another experiment, not shown, it was 0.6% and 0.7% in samples 1 and 2, respectively. In the latter experiment, 39% and 58% of the pro-
teinase was recovered in the parasite fraction at the two sampling times. In the Aotus monkey, the reticulocyte count was 1.2% and 0.6%
for the two samples in Experiment 1, and 1.2% in Experiment 2.

with the remainder of the ghost fraction. The wash fluids from the ghosts and parasites were added to the haemolysate. The first centrifugations of the ghost fraction yielded additional parasites, and this process was repeated until a parasite-free ghost fraction was obtained.

The specific activity of acid protease of infected Rhesus red cells was about 2.5 times that of normal cells (Table 1). The parasite-rich fraction was greatly enriched in acid proteinase, the specific activity being about 100 times that of the infected cells. About 35% to 40% of the enzyme, and 0.5% or less of the cell protein was associated with the parasites. In a second experiment (not shown), 39% and 58% of the proteinase appeared in the parasite fraction in the two stages. There was little difference in specific activity between stage 1, which contained 40% ring stages and 40% early trophozoites, and stage 2 which contained 47% early trophozoites and 42% late trophozoites.

Acid phosphatase, which is often present in the same subcellular compartment as acid proteinase, was found primarily in the haemolysate, with less than 5% appearing in the parasite fraction. The specific activity was similar in infected and non-infected cells.

The Aotus-*P. falciparum* system differed somewhat from the *P. knowlesi*-infected red cells in that there was little difference in specific activity between normal and infected cells. Also, there seemed to be a greater variability in the distribution of proteases, 71% of the enzyme being localised in the parasites in one case. The difference could be due to leakage of enzyme, as the parasitaemia was similar in the two experiments. Again, very little of the protein of the infected cells was present in this fraction, so that there was a great enrichment of the proteinase in the parasites.

To determine if the parasites and the host cells contain distinct proteases, the *p*H response and the susceptibility to inhibitors of the enzyme from each source were tested. Maximal activity for the *P. knowlesi*-enzyme occurred at *p*H 3.6. There was considerable activity between *p*H 3.0 and 4, and almost none at *p*H 2 (Fig 1). In contrast, the host cell exhibited maximal activity at *p*H 2 to 2.5, and was relatively inactive at *p*H 3.5 and above. The parasites also had a reasonably active alkaline protease. Ghosts from infected cells show a *p*H response similar to that of ghosts from normal cells.

Maximal protease activity in *P. falciparum* also occurred at *p*H 3.6, while that of the enzyme from host cell ghosts occurred at *p*H 2.5. In this case, however, there was little difference in activity between *p*H 2.5 and 4 in both ghosts and parasites. Again, there was some activity in the alkaline range.

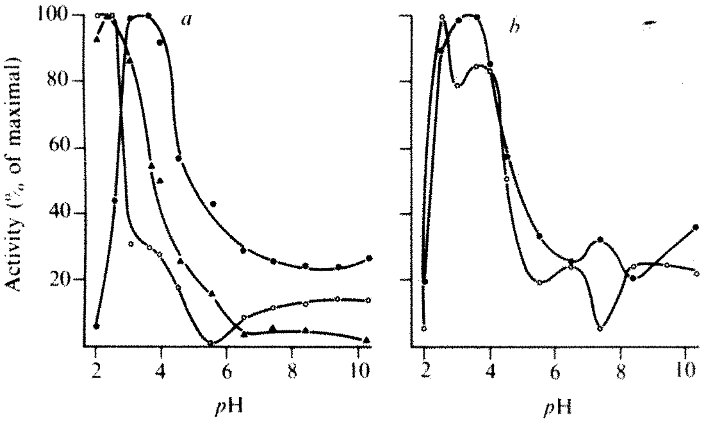

Fig. 1 *p*H response of proteases from parasites and host cell ghosts. *a*, Rhesus monkey; ○, RBC ghosts; ▲, ghosts of infected cells. ●, *P. knowlesi*. *b*, ○, RBC ghosts, Aotus monkey; ●, *P. falciparum*, FVO strain. Buffers: Glycine-HCl, *p*H 2–3; NaAcetate-acetic acid, *p*H 3.6–5.5; potassium phosphate, *p*H 6.5–7.4; Borate-NaOH, *p*H 8.4–10.3. Specific activities: *P. knowlesi*, 2560; Rhesus ghosts. 184; Rhesus ghosts from infected cells, 340; *P. falciparum*, 2210; Aotus ghosts, 164.

A number of specific and very potent proteinase inhibitors have been isolated from cultures of actinomycetes[16–19], and these have been of value in characterising proteases of various mammalian cells[20–22]. Thus, pepstatin inhibits cathepsin D in submicrogramme quantities, leupeptin and chymostatin are potent inhibitors of cathepsin B, while antipain is most active against cathepsin A[18]. These compounds are also potent inhibitors of various extracellular proteases.

Table 2 Concentration of Inhibitors Giving 50% Inhibition of Acid Proteinase

	P. knowlesi	Rhesus ghosts	*P. falciparum*	Aotus ghosts
		(μg ml^{-1})		
Pepstatin *	0.010	<0.0008	0.002	<0.002
Chymostatin	0.10	~0.005	0.020	~0.0025
Antipain	5.0	~0.5	1.0	~0.20
Leupeptin	~30	~5.0	2.5	~0.3

* The concentrations indicated for pepstatin on the ghosts were the lowest tested and produced more than 95% inhibition.

Pepstatin was the most potent inhibitor against the protease of both species of parasites and ghosts (Figs 2, 3, Table 2). Complete inhibition of the protease from red cell ghosts from Rhesus and Aotus monkeys occurred at 0.8 and 2 ng ml, respectively, the lowest concentrations tested. The parasite proteases were also very sensitive to pepstatin, 50% inhibition occurring at 10 ng ml^{-1} and 2 ng ml^{-1}, respectively, for *P. knowlesi* and *P. falciparum*. A small percentage of the activity in each species was insensitive to the inhibitor.

Chymostatin also was a potent inhibitor of protease activity in both ghosts and parasites. In both species of ghosts, there was a rapid transition from complete to no inhibition, and 50% inhibition occurred at about 5 and 2.5 ng ml^{-1} for the enzyme from Rhesus and Aotus ghosts, respectively (Table 2). The enzymes from the parasites were inhibited at somewhat higher concentrations, and the change in degree of inhibition was more gradual than in the ghosts.

Antipain and leupeptin also inhibited protease activity, but at somewhat higher concentrations than pepstatin and chymostatin. Again, the transition from complete to no inhibition was very sharp in the ghosts and more gradual in the parasite, and the enzymes from the ghosts were the more sensitive.

The inhibition of the parasite enzyme was similar at *p*H 3 and 3.9 (Fig. 2), and tripling the amount of enzyme did not affect the degree of inhibition. None of these compounds inhibited the alkaline protease (Table 3) at the highest concentrations tested.

The acid proteinase of the parasites was not greatly affected by iodoacetamide or *p*-chloromercurobenzoate (Table 3). The insensitivity to these compounds, and lack of activation by dithiothreitol suggest that the major enzyme lacks essential sulphydryl groups. Suramin, an antitrypanosomal drug that accumulates in lysosomes and inhibits proteolysis within these particles[23] also was without effect, as was phenylmethanesulphonyl fluoride, an inhibitor of serine proteases. None of these compounds had any effect on the alkaline protease, except perhaps high concentration of dithiothreitol.

These data indicate that both *P. knowlesi* and *P. falciparum* have an acid proteinase that degrades haemoglobin optimally at a *p*H of 3–3.6. We have found that *P. berghei* contains a similar enzyme, with optimal activity at *p*H 2.5 to 3 (ref. 8). The existence of an active acid proteinase in the malarial parasite had not been previously reported. Cook *et al.*[7], using denatured globin as substrate, found that *P. berghei* and *P. knowlesi* contained an acid proteinase that acted optimally at *p*H 4 and 5, respectively. But the maximal activity in both species occurred at *p*H 8, and the acid proteinase lost most of its activity within 12 h at 2° C. We have found that haemoglobin is degraded much more slowly in the alkaline range in both species studied here, and that *P. berghei* exhibits almost no activity towards haemoglobin above *p*H 6.5[8]. We have also found the acid proteinase to be relatively stable. Further evidence that our enzyme differs from that of Cook *et al.* is the sensitivity of their enzyme to EDTA.

The data here also differ in certain respects from our findings on the *P. berghei*-mouse system[8]. In the latter, the specific proteinase activity of infected cells is five to ten times that of the normal cells. Interestingly, the specific activity of normal mouse red cells is about fifteen-fold higher than that of either monkey, while the activity of infected cells is thirty to seventy-five times that of the infected primate cells. The distribution of acid phosphatase between the haemolysate, ghosts and parasites differs greatly from that of the acid proteinase, and suggests that the parasites have little of the phosphatase. Such a conclusion would be consistent with results of a recent cytochemical study, in which bodies positive for acid phosphatase were found in the host cell but not the parasite[24]. Our procedure for obtaining parasites could, however, lead to leakage of enzyme.

The *p*H response curves indicate that both *P. knowlesi* and *P. falciparum* contain an acid proteinase that is distinct from that of the host red cell. This is especially clear in the Rhesus-*knowlesi* system, where the enzyme from the host cell has a very low *p*H optimum. It is of interest that the *p*H response of the two species of monkeys differs, the Aotus enzyme exhibiting very little activity at *p*H 2, while the Rhesus enzyme is fully active at this *p*H.

Recent studies have shown that some enzymes attributed to the parasite may be of reticulocyte origin[25]. But it seems unlikely that the 'parasite' proteases described here are due to contamination by reticulocytes. In the case of the *P. knowlesi*-Rhesus monkey system, the percentage of reticulocytes (Table 1) was 0.9%. In a second experiment, the percentages for samples 1 and 2 were 0.6% and 0.7% respectively. In this experiment, 39% and 58% of the acid protease appeared in the 'parasite' fraction. In the experiments on *P. falciparum* the percentage of reticulocytes ranged from 0.6% to 1.2%, and there was no correlation between the reticulocyte level and the percentage of enzyme appearing in the parasite fraction. It may also be noted that the per cent of both total protein and acid phosphatase that appears in the parasite fraction is extremely low.

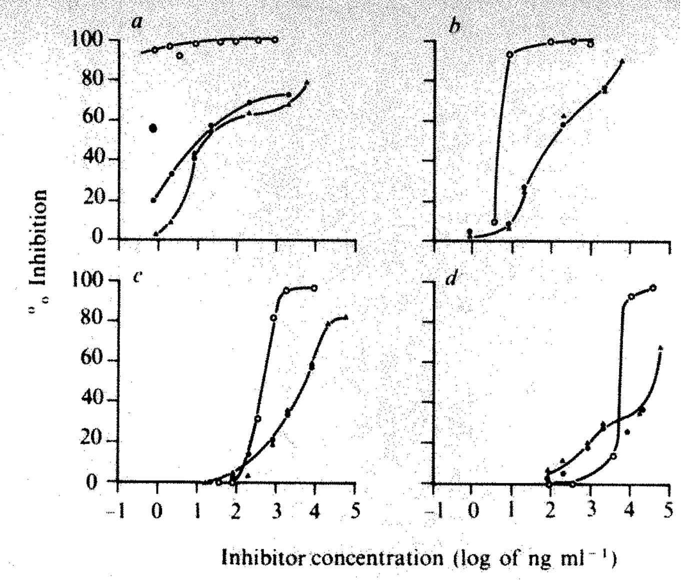

Fig. 2 Effects of inhibitors on acid proteases of *P. knowlesi* and ghosts of Rhesus red cells. *a*, Pepstatin; *b*, chymostatin; *c*, antipain; *d*, leupeptin. ○, Rhesus red cell ghosts, tested at *p*H 2.5; ●, *P. knowlesi*, tested at *p*H 3.0; ▲, *P. knowlesi*, tested at *p*H 3.9.

The *p*H response of the protease from the ghosts and parasites also argues against a possible reticulocyte origin (Fig. 1). Thus, at *p*H 2.0, the enzyme from Rhesus red cells is fully active, while the *P. knowlesi* enzyme has virtually no activity. For the 'parasite' enzyme to be from reticulocytes, it would not only be necessary for the reticulocytes to be completely devoid of the erythrocyte protease, but it would also imply that the erythrocytes are capable of synthesising a new enzyme that is distinct from that found in reticulocytes.

The results with the inhibitors from actinomycetes are of interest and also suggest that the parasites and red cell ghosts contain distinct enzymes. All of the inhibitors are more effective against the parasite protease, the concentration giving 50% inhibition usually differing by an order of magnitude or more. The sensitivity to pepstatin and chymostatin of the ghosts enzyme appear to be the greatest yet reported, as

Table 3 Effects of Various Compounds on Protease Activity of Red Cell Ghosts and Parasites

		Activity (% of control)					
		P. knowlesi		Rhesus ghosts	*P. falciparum*		Aotus ghosts
		*p*H 3.9	*p*H 10.3	*p*H 2.5	*p*H 3.0	*p*H 10.3	*p*H 10.3
Phenylmethanesulphonylfluoride	0.5 mM	103	90	90	92	97	105
	1.5 mM	—	90	—	81	98	97
Iodoacetamide	1.0 mM	90	—	—	—	—	—
	2.0 mM	—	90	96	101	102	99
	5.0 mM	—	96	95	98	113	98
p-Chloromercurobenzoate	0.5 mM	84	94	97	90	105	—
	1.0 mM	—	90	94	81	106	106
EDTA	2.0 mM	95	91	100	116	105	—
	10 mM	—	91	—	103	107	93
Dithiothreitol	1.0 mM	108	116	100	99	104	93
	5.0 mM	—	61	—	92	105	74
Suramin	0.5 mM	90	—	—	—	—	—
Pepstatin	2 μg ml^{-1}	—	96*	—	—	—	—
Chymostatin	2 μg ml^{-1}	—	96*	—	—	—	—
Antipain	8 μg ml^{-1}	—	107*	—	—	—	—
Leupeptin	20 μg ml	—	109*	—	—	—	—

* Tested at *p*H 9.3.

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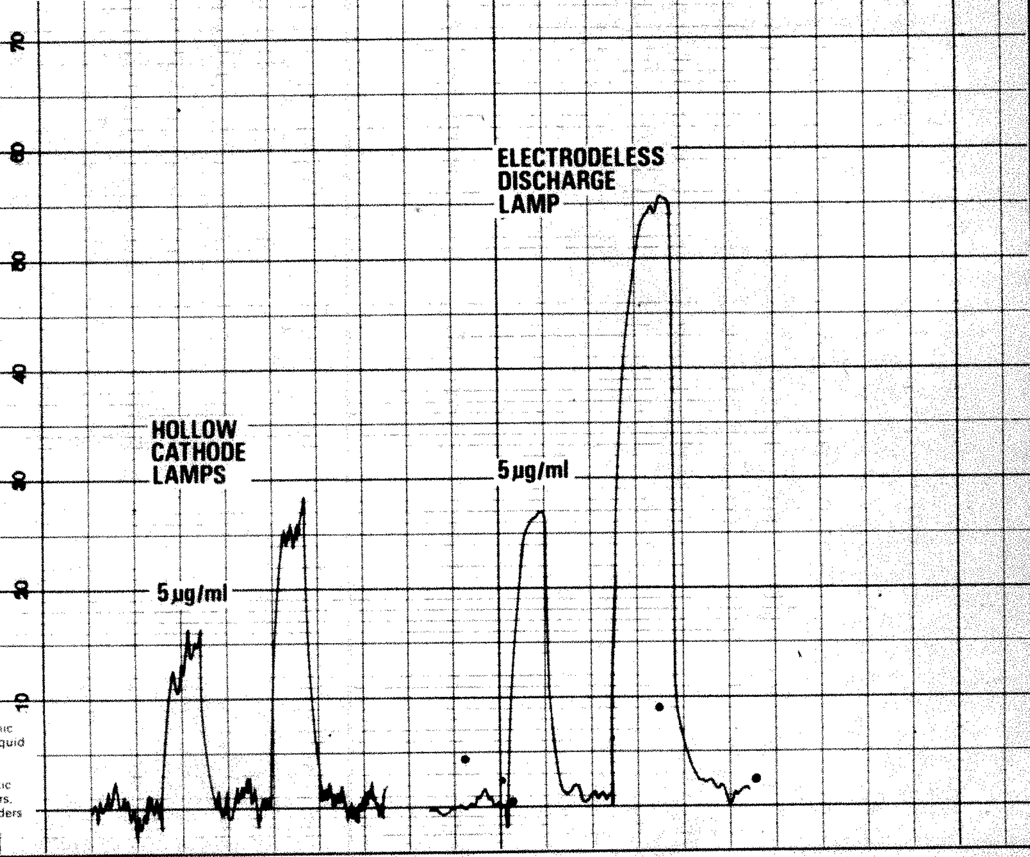

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nanogramme quantities cause complete inhibition (recently we have found the sensitivity to these inhibitors of partially purified enzyme from *P. berghei* to be even greater than the enzymes reported here). The molar ratio of enzyme to inhibitor is, however, important in determining the sensitivity[2].

In view of their reported specificity[18,21], it is surprising that each of the inhibitors is capable of inhibiting virtually all the protease activity. Thus, while pepstatin inhibits acid proteases at very low concentrations, it reportedly does not inhibit any of the proteases inhibited by chymostatin (chymotrypsin, cathepsin B), antipain (trypsin, papain, cathepsin A), or leupeptin (papain, cathepsin B)[16–20]. Similarly, the other three inhibitors reportedly do not inhibit acid proteases, except at concentrations much greater than those used here. Thus, the red cells and parasites each contain a protease that is unusual in being susceptible to all of the inhibitors.

Our data suggest that the major acid proteinase of both the parasites and the ghosts is like cathepsin D. This is based on the *p*H optima, the great sensitivity to pepstatin, and the lack of sensitivity to the sulphydryl reagents or the serine protease inhibitor (PMSF) tested in Table 3.

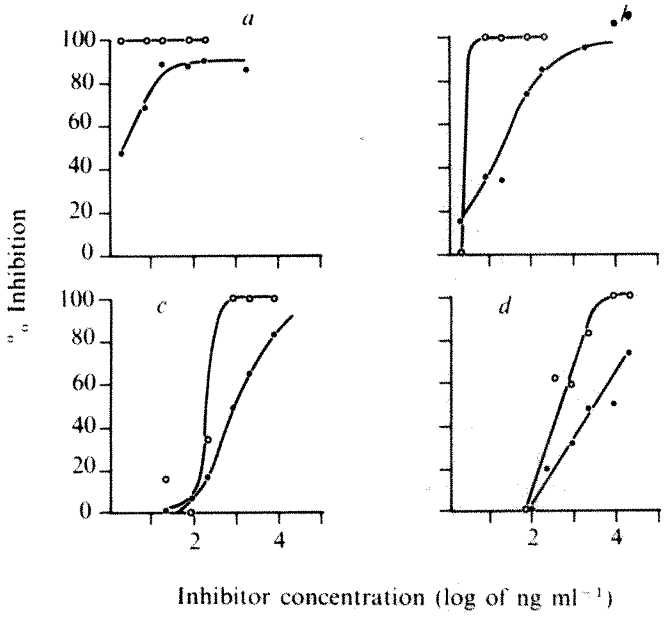

Fig. 3 Effects of inhibitors on acid proteases of *P. falciparum* and ghosts of Aotus red cells. *a*, Pepstatin; *b*, chymostatin: *c*, antipain; *d*, leupeptin. ○, Aotus red cell ghosts, tested at *p*H 2.5; ●, *P. falciparum*, FUP strain, tested at *p*H 3.0.

It will be of interest to determine the susceptibility to the malarial proteases of haemoglobins of various species. *P. falciparum* can infect Aotus monkeys, which contain a human type haemoglobin[26], but cannot invade Macacus monkeys, which do not[27]. The ability of a given species of parasite to infect a particular species of organism could perhaps be in part determined by its ability to degrade the haemoglobin of the host red cell. The marked difference in the *p*H-response curve of the enzyme from the red cell ghosts of Aotus and Rhesus monkeys is of interest in this respect.

Finally, we should like to suggest that protease inhibitors may be of value in inhibiting the growth of the malarial parasite by selectively acting on a process that is essential to the parasite but not, at least over a short period, to the cells of the host. The erythrocytic stages of the parasite apparently must obtain nutrients by degradation of host cell haemoglobin, and also apparently must degrade many of its own organelles. Therefore, it seems to be very dependent upon the action of intracellular proteases. Protease inhibitors would perhaps be more useful than drugs that inhibit macromolecular synthesis in all eukaryotic cells since the former are directed specifically at a metabolic difference between the host and the invading cells. In this respect, preliminary experiments show at least one of the protease inhibitors, pepstatin, greatly inhibits the growth of the parasite in an *in vitro* culture system.

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Prostaglandin E_2 as stimulator of haemopoietic stem cell proliferation

ALTHOUGH a strict correlation between prostaglandins and the cyclic AMP system has been suggested for several cell types, and the mitogenic effect of cyclic AMP has also been proved[1,2], the mitotic effect of prostaglandins has been demonstrated only for some cell systems[3]. Also, little is known about the haemopoietic effect of prostaglandins. As proved by Dukes[4], several prostaglandins, especially PGE_2, enhance the ^{59}Fe and ^{14}C-glucosamine incorporation into bone marrow cells and potentiate the effect of erythropoietin, *in vitro*. We have studied the effect of PGE_2 on the proliferative state of haemopoietic stem cells (spleen colony forming units:CFU).

In the normal steady state by far the greater part of the pluripotent stem cell population is either not in cell cycle (G_0 state[5]) or is passing through a rather long cell cycle. Stem cells, however, may be triggered into the cell cycle by imposed stimuli for differentiation or regeneration *in vivo* or by certain drugs such as steroids[1] *in vitro*.

The proliferative state of CFU can be well characterised by their *in vitro* sensitivity to ^{3}H-thymidine of high specific activity[6]. In the normal steady state less than 10% of the CFU population is sterilised by 30 min exposure to ^{3}H-thymidine.

The test system used was almost identical with that described by Byron[1]. Bone marrow was aspired from the femora of 12–14 weeks old Balb/c × CBA/F_1 hybrid male mice and suspended in Fischer's medium (Gibco, New York). One millilitre of this bone marrow suspension (containing $3.1 \times 10^6 - 4.2 \times 10^6$ nucleated cells) was incubated in siliconised bottles, in an atmosphere containing 5% CO_2, at 37° C in a shaking water bath. Various concentrations of PGE_2 (Chinoin, Budapest) were added to the cultures at the beginning of the incubation. The stock solution contained 2–5,000 μg ml^{-1} in 96% ethanol. PGE_2 dilutions were always freshly prepared in Fischer's medium and added to the cultures in a volume of 0.1 ml. The ethanol concentration of the cultures never exceeded a final concentration of 0.05%. The same quantity of ethanol was added to the control samples. The number of CFU in S phase was measured according to Becker *et al.*[6]. After incubation with various concentrations of PGE_2 for 2.5 h, 200 μCi per 0.2 ml of pre-warmed, isotonised ^{3}H-thymidine (21–28 Ci mmol^{-1}, Radiochemical Centre, Amersham), was added to half of the cultures. After 30 min incubation with ^{3}H-thymidine the bone marrow suspensions were diluted with ice-cold Hanks' solution to the required cell concentration and were transplanted immediately into 10–10 lethally (850 R ^{60}Co) irradiated recipients. The spleen colony count was determined 9 d after transplantation[7]. The difference in CFU count between cultures incubated with or without ^{3}H-thymidine was a measure of the number of CFU in S phase.

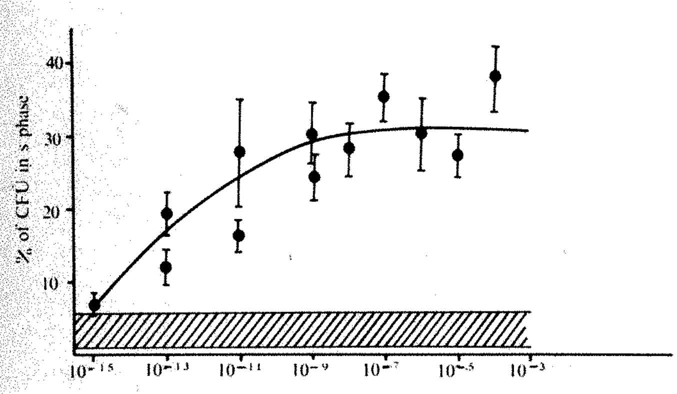

FIG. 1 Effect of PGE_2 on the percentage of CFU in S phase, *in vitro* measured as the percentage of CFU sterilised by 30 min exposure to ^{3}H-thymidine. Each point represents the mean and standard deviation of one experiment. Hatched over control values.

The effect of PGE_2 on CFU proliferation was studied in the dose range 10^{-4}–10^{-15} M (Fig. 1). A stimulatory effect could be clearly demonstrated at 10^{-13} M and reached its plateau at 10^{-9} M. No cytotoxic effect was found even at the highest concentration studied.

Since it has been proved that cyclic AMP may trigger the entry of CFU into the cell cycle *in vitro*[1] and prostaglandins may enhance cyclic AMP production in certain cells[2,8,9], we next studied whether the PG effect could be inhibited by decreasing the intracellular cyclic AMP level. Imidazole increases the enzymatic destruction of cyclic AMP, consequently suppressing the effect of agents acting through cyclic AMP[10]. The effect of 10^{-6} M PGE_2 was studied in the presence of 4 × 10^{-3}M imidazole. The culturing system was identical with that described above. Neutralised imidazole was added to the cultures at the start of incubation. Table 1 shows that imidazole had no effect on PGE_2-induced increase in the number of CFU in S phase.

TABLE 1 Effect of imidazole on PGE_2-induced increase in number of CFU in S phase

	CFU/6 × 10^4 cells		
	Incubated without ^{3}H-thymidine*	Incubated with ^{3}H-thymidine*	Killing effect %
Control	11.25 ± 2.66	10.77 ± 3.05	4.0
	12.87 ± 2.16	12.14 ± 2.87	6.0
PGE_2 (10^{-6} M)	11.12 ± 1.56	7.25 ± 2.95	35.0†
	12.67 ± 2.96	6.60 ± 1.36	48.0†
	12.50 ± 2.30	9.74 ± 1.76	28.0‡
PGE_2 (10^{-6} M) + Imidazole (4 × 10^{-3} M)	10.50 ± 1.96	7.22 ± 1.48	31.0†
	11.10 ± 1.66	5.60 ± 1.02	49.0†
	11.90 ± 2.10	8.66 ± 1.87	27.0†

* Mean ± s.d.
† $P < 0.01$
‡ $P < 0.05$

The experiments reported suggest PGE_2 to be a potent nontoxic stimulator of the proliferation of haemopoietic stem cells *in vitro*. The effective PGE_2 concentrations *in vitro* (10^{-13} M to 10^{-4} M, that is, 0.035 pg ml^{-1} to 35 × 10^6 pg per ml^{-1}) fall in the range of PGE level proved to be present in human serum (316 ± 36 pg ml^{-1})[11]. If administered in higher concentrations (minimum 10^{-5} μg per g body weight), though still within the physiological range, this triggering effect on CFU can also be demonstrated *in vivo* (unpublished data).

The cell cycle action of PGE_2 on haemopoietic stem cells is through some mechanism different from cyclic AMP since it cannot be prevented by imidazole. This effect of PGE_2 resembles the stimulatory effect exerted by testosterone on CFU proliferation which also does not act through the cyclic AMP system[12].

Summarising the previously published data[3,8,13] and the present experiments, the correlation among PG, cyclic-AMP-system and cellular proliferation seems to differ according to cell types. (1) Prostaglandin enhances the intracelluluar cyclic AMP level and mitotic activity in thymocytes[3]. (2) It may induce cyclic AMP accumulation in lymph node cells too, without causing their blast-transformation or mitosis.[8] (3) PGE_2 triggered resting (G_0) haemopoietic stem cells into the cell cycle even if increased cyclic AMP destruction had been induced. (4) Finally, PGE may restore controlled growth of transformed cells[13] through the cyclic AMP system.

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Immunosuppressive Effect of an Anti-interferon Serum

INTERFERON has been reported to play an important role in immune responses[1-6]. Using interferon[3,5,6] acceleration of mouse skin allograft rejection has been demonstrated in addition to an increase in the cytotoxic action of lymphocytes on appropriate target cells *in vitro*. It has also been shown that prolongation of skin allografts can be achieved by the use of anti-interferon serum[3]. To further test the possibility of involvement of interferon in immune responses an attempt is here described to block the interferon effect by anti-interferon serum.

Anti-interferon serum was obtained by immunisation of a goat with the interferon induced in L cell culture by the Newcastle disease virus (NDV). The titre of the crude preparation was 800 to 1,200 units ml^{-1}. Before immunisation, blood was collected from the goat and the control serum was prepared. The animal received forty-nine weekly subcutaneous injections of the crude interferon. Later interferon preparations were purified and concentrated before use.

Initially purification and concentration were as follows: liquid containing interferon was centrifuged at 18,000 r.p.m. for 20 min and then concentrated ten-fold using polyethylglycol (molecular weight 40,000). Subsequently the interferon was purified by passage through Millipore filters of 1.2 µm or 0.45 µm diameter and concentrated by UM-2 (Amicon) filtration. The titre of the interferon sample obtained in this way was 6,000 to 8,000 units ml^{-1}. Goats were thus given 60,000 to 80,000 units of interferon in a single subcutaneous injection. After twenty-two injections the goat serum revealed antibodies to the interferon.

The serum was stored at $-30°$ C. Before use it was adsorbed with an L-cell suspension (3×10^6 cells ml^{-1} serum) at 37° C for 1 h and then at 4° C for 24 h and similarly with lymphocytes of C57Bl/6 mouse lymph nodes (3×10^6 cells ml^{-1} serum). The sera were then tested in a microcytotoxicity test and showed no toxic activity on L cells or lymphocytes.

For titration of anti-interferon serum, a series of two-fold dilutions of control and immune sera from 1 : 2 to 1 : 256 in TC 199 supplemented with 2% inactivated bovine serum was made. To each serum dilution, 8 units of the interferon were added in an equal volume and the mixture was incubated at 37° C for 1 h and at 4° C for 24 h. Then the contents of each tube were placed on the L cell monolayer (the cells had been seeded the day before) and incubated at 37° C for 18 to 20 h, whereupon 100 TCD$_{50}$ of the test-virus (VSV) was added. Results were evaluated 24 h after the challenge with the virus. The titre of the anti-interferon serum was expressed as the maximum dilution which still neutralised the protective effect of the interferon. The anti-interferon serum employed showed a titre of 1 : 64. The control serum did not reveal any protective activity.

Four groups of female C57Bl/6 mice were used. Mice of group I received a single injection of 7×10^6 L cells subcutaneously in each of eleven sites and, simultaneously, 0.3 ml of anti-interferon serum intraperitoneally. This volume of anti-interferon serum was administered daily for 5 d in all. Group II was immunised with L cells and the control serum was given using the same time course of injections. Group III was similarly immunised with L cells only and group IV remained intact.

Table 1 Cytotoxic Action of C57Bl/6 Mice Lymph Node Lymphocytes on L Target Cells.

Immunisation			
L cells + anti-interferon serum	L cells + normal serum	L cells	Intact
(I)	(II)	(III)	(IV)
97,765 ± 6,490*	59,994 ± 3,850	38,885 ± 4,730	143,319 ± 9,900

* Mean number of viable L cells ml^{-1}.

At 7 to 10 d after the onset of immunisation, lymph nodes were taken from all the mice and the cytotoxic activity of the lymphocytes was investigated. Suspensions of lymphocytes were added to the 24 h monolayer of L target cells in the ratio proportion of 50 : 1. After 48 h incubation, cells were collected by treatment with 0.25% trypsin and their viability determined by staining with eosin-trypan blue. Statistical significances were estimated by the Student's *t* test.

As seen from Table 1, in mice given anti-interferon serum in addition to L cell immunisation (group I) lymphocyte cytotoxicity to L target cells was suppressed as compared with that of lymphocytes from mice given normal goat serum (group II) ($P<0.01$).

The sera of experimental and control groups were tested in parallel in a microcytotoxicity test with undiluted guinea pig complement[7]. Terasaki plates (well size 10 µl) were used. The serum (1 µl) was incubated with L cells (1,000 to 1,500 cells per well). After 30 min incubation at 37°, 5 µl of the complement (undiluted guinea pig serum) were added and the mixture was incubated for an additional 1.5 h. The supernatant was then shaken from the wells and supravital stain was added. After 3 min exposure, the stain was removed and cells were counted. The reaction was evaluated as follows: −, 0 to 15% of dead cells; ±, 16 to 20% of dead cells; +, 21 to 30% of dead cells; 2+, 31 to 50% of dead cells; 3+, 51 to 80% of dead cells; 4+, more than 81% of dead cells.

As seen in Fig. 1, the sera of mice immunised with L cells, and those of the animals immunised with L cells and given normal goat serum, showed a cytotoxic activity to L cells in a titre of 1 : 40. In contrast, the sera of mice immunised with L cells plus anti-interferon serum did not reveal any cytotoxic activity.

It has therefore been demonstrated that the administration of anti-interferon serum can induce an immunosuppressive effect

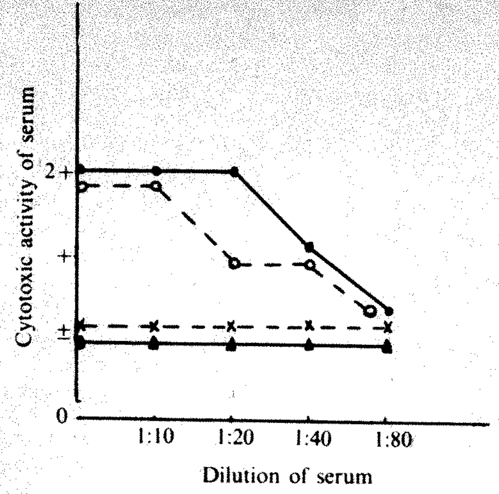

Fig. 1 Cytotoxic action of C57Bl/6 mouse serum on L target cells. ●, Mouse serum after L cell immunisation; ○, mouse serum after L cell immunisation and administration of control serum; ×, mouse serum after L cell immunisation and administration of anti-interferon serum; ▲, control untreated mouse serum.

which is revealed both at the level of cellular and humoral immunity. A certain decrease in the cytotoxicity of lymphocytes of mice receiving normal goat serum may be accounted for by the concurrent action of the serum with the immunising agent (L cells). On these grounds it seems possible that interferon is a prerequisite for the full expression of the response to an antigenic stimulus. Also, antigens on this basis are interferogens[4]. We therefore believe that interferon may play a part in the recognition of a specific antigen by an immune lymphocyte and may be a necessary factor involved in the interaction between the antigen and a specific effector cell or antibody.

The exact manner in which interferon is involved in the immune process is not indicated in the present experiments, but it is intriguing to contemplate the possibility that autoimmune (allergic) diseases are connected with the abundant interferon production and anti-interferon serum might be used for the treatment of autoimmune disease. It is also our belief that the immunosuppression which is sometimes observed in cancer patients and which has, by some authorities, been thought responsible for the malignant conditions, is associated with inhibition of interferon synthesis.

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In vitro assembly of 30S ribosomal particles from precursor 16S RNA of *Escherichia coli*

THE concept of self-assembly has been shown to apply to the assembly of the *Escherichia coli* 30S ribosome *in vitro* by Nomura and his co-workers[1,2]. The reconstitution of functional 30S ribosomes from purified mature 16S RNA (m16 RNA in the nomenclature of Hecht and Woese[3]) and total 30S proteins has been studied in detail. The macromolecular information necessary for this *in vitro* assembly clearly resides in the component parts with no requirements for enzymes or cellular structures (such as membranes) which are not ultimately part of the 30S ribosome. The reconstitution system developed by Traub and Nomura[1] has been useful in a variety of studies which have greatly aided our understanding of ribosome assembly, structure, and function. The biosynthesis of ribosomes, however, is known to differ in several ways from this *in vitro* reconstitution. The *in vivo*

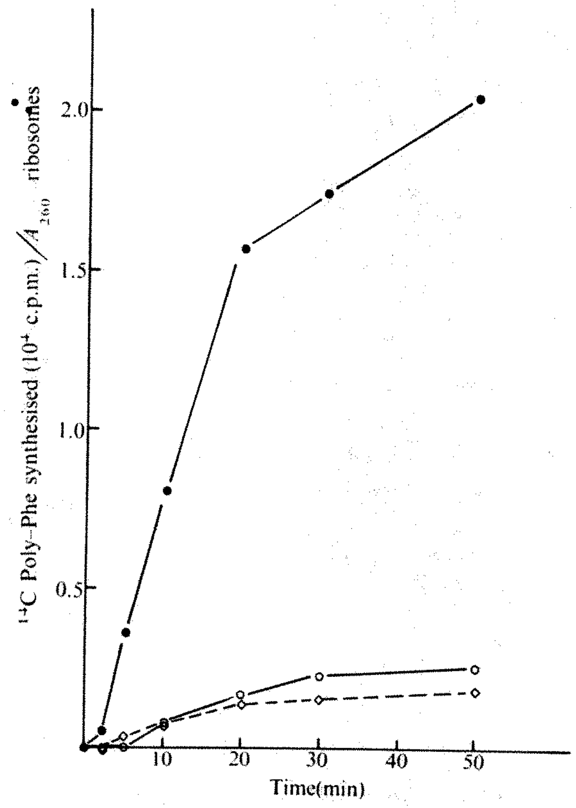
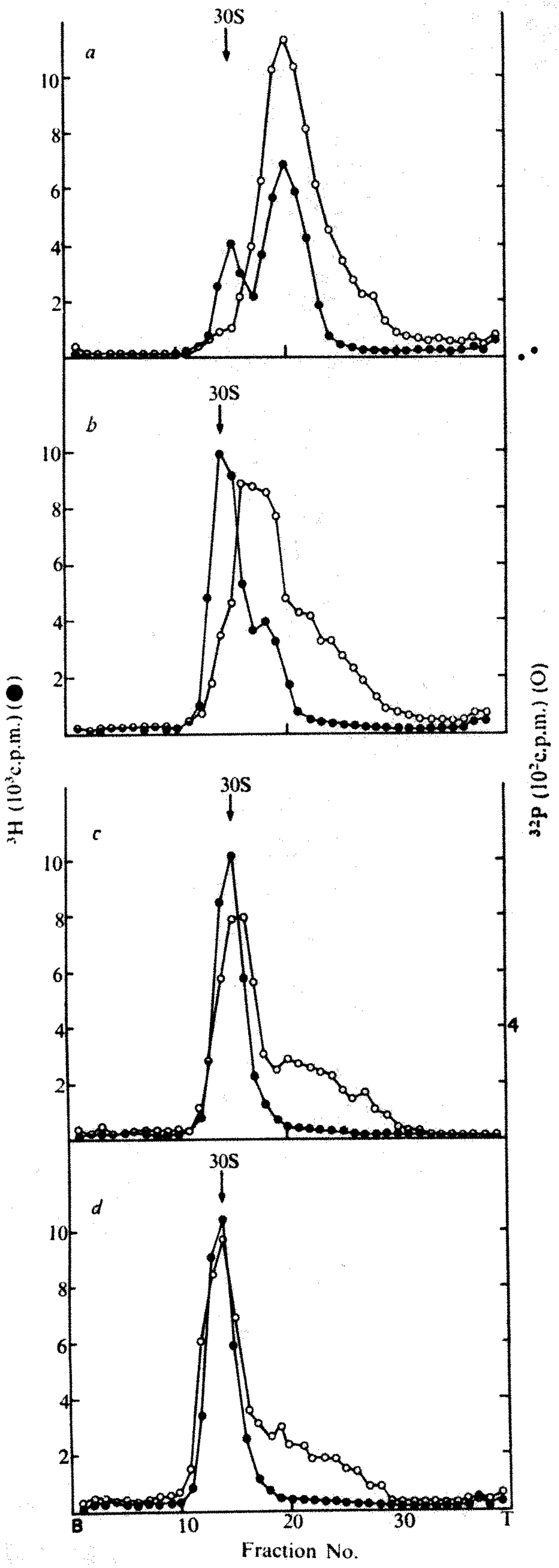


FIG. 1 Kinetics of incorporation of ^{14}C-phenylalanine into hot TCA-precipitable peptide. A 1.25 ml reaction mixture, prepared as described[6], was initiated at 37° C with poly (U). The 30S ribosomes derived by reconstitution were mixed with mature 50S ribosomes. Reconstituted ribosomes were prepared as described[6] using m16 RNA and p16 RNA. The RNA's were prepared from a culture of *E. coli* D10 (Met-RC relaxed) which had been deprived of methionine for 90 min. The m16 RNA was labelled by incorporating ^{3}H-uracil during the exponential growth phase. The p16 RNA was labeled with $^{32}PO_4$ during the period of methionine deprivation. RNA was extracted from the cells with SDS and phenol and applied to a 5–20% sucrose gradient. The 16S region was recovered and the RNA was precipitated with ethanol. The RNA was then dissolved in Tris-HCl buffer and applied to 3.2% polyacrylamide gels with a diameter of 20 mm. The conditions of electrophoresis were described by Bishop *et al.*[14] The peaks of m16 and p16 RNA were recovered after the gels had been sliced and eluted into buffer. Appropriate fractions were pooled to give a p16 RNA preparation that had 9% m16 RNA present. There was no detectable p16 RNA in the m16 RNA preparation. The poly (U) assays contained 0.45 A_{260} units of 30S ribosomes. ●—●, 30S particles containing m16 RNA; ○—○, 30S particles containing p16 RNA; ◇—◇, 9% of the activity of m16-containing particles.

process is composed of a series of cooperative and sequential interactions between the primary ribosomal RNA transcripts and of ribosomal proteins, as well as the modification of both RNA and protein[4,5].

We have attempted to assess the role that precursor 16S RNA (p16) might play in the *in vivo* assembly of ribosomes by carrying out reconstitution experiments with this form of RNA and the proteins derived from mature ribosomes. We chose the unmethylated precursor which accumulates during methionine deprivation of a relaxed auxotroph of *Escherichia coli*. Using purified p16 RNA or a differentially labelled mixture of m16 and p16 RNA, we have shown that p16 RNA assembles to a homogeneous 30S particle containing all the ribosomal proteins except S3 (and possibly S6) and that such particles are inactive in a poly (U) incorporation assay (unpublished).



In this report, we have examined the possibility that P16 RNA might be preferred to mature 16S RNA (m16) in the reconstitution process, and that the use of p16 RNA might lower the unusually high activation energy found for the assembly 30S particles with m16 RNA[2]. Total 30S ribosomal proteins and p16 RNA, under the conditions described by Traub *et al.*[6] form a particle which co-sediments with native 30S ribosomes (see Fig. 2*d*). There is no poly (U) directed biosynthesis of polyphenylalanine attributable to the particles made with p16 (Fig. 1).

The p16 RNA was competed with m16 RNA in the reconstitution system to determine which was the preferred substrate for the interaction with proteins. When the amounts of total 30S proteins were limiting (Fig. 2, *a–c*) the m16 RNA was preferentially assembled into particles when both p16 and m16 RNA were present. Only at higher concentration of protein did the p16 RNA become incorporated into a homogeneous particle. Thus, in this system, m16 RNA is clearly more efficient than p16 RNA in interacting with proteins and forming an RNP particle.

We examined the possibility that p16 RNA might lower the activation energy for the reconstitution process by carrying out the reaction at various temperatures. Because particles with p16 RNA have no protein synthesizing capacity, we had to devise an alternative method for measuring the end point of the assembly. Both p16 and m16 RNA are highly sensitive to pancreatic RNase, but when assembled into RNP particles, become relatively resistant. Therefore, the temperature-dependent acquisition of RNase resistance was used as a measure of the overall reconstitution process for both types of RNA. As Fig. 3 shows, there is no discernable difference between the RNAs in the temperature dependence of their reconstitution into particles. Therefore, p16 RNA does not have a lower activation energy for assembly as measured by the thermal energy required to give complete 30S particles when the reconstitution is carried out with excess proteins.

There are at least two major differences between the p16 and m16 RNAs. The precursor rRNA contains excess olignucleotides at both its 3′ and 5′ ends and also lacks all of the fifteen or so methyl groups found in m16 RNA[7–10]. The relative contribution of the methylated sequences of m16 RNA

FIG. 2 Competition between m16 and p16 RNA for limiting 30S proteins. Reconstitution reactions with 0.2 A_{260} units of a mixture of ^{3}H-uracil-labelled m16 RNA (●—●) and ^{32}P labelled p16 RNA (○—○) were incubated with increasing amounts of total 30S proteins. The reconstitution reactions were done, as previously described[6] at 40° C for 20 min. Each reaction was cooled to 0° C and layered directly on 5 ml 10–30% tris-Mg^{2+} sucrose gradients (10 mM Tris-HCl, *p*H 7.8 and 0.3 mM MgCl$_2$). The gradients were run 3 h at 49,000 r.p.m. in a SW 50.1 rotor. The gradients were pumped out with an Isco density gradient fractionator and the radioactivity in each fraction of the gradient was determined by liquid scintillation spectroscopy. *a*, 23 μg 30S protein per A_{260} unit of mixed 16S RNA; *b*, 35 μg protein; *c*, 46μg protein; and *d*, 69 μg protein. In this experiment a portion of the ^{32}P label is slow sedimenting and does not reconstitute to a 30S particle. Other experiments with this variable RNA fraction have shown that it contains internal nucleolytic cleavages (by melting experiments) and probably represents mRNA or degraded p16 RNA.

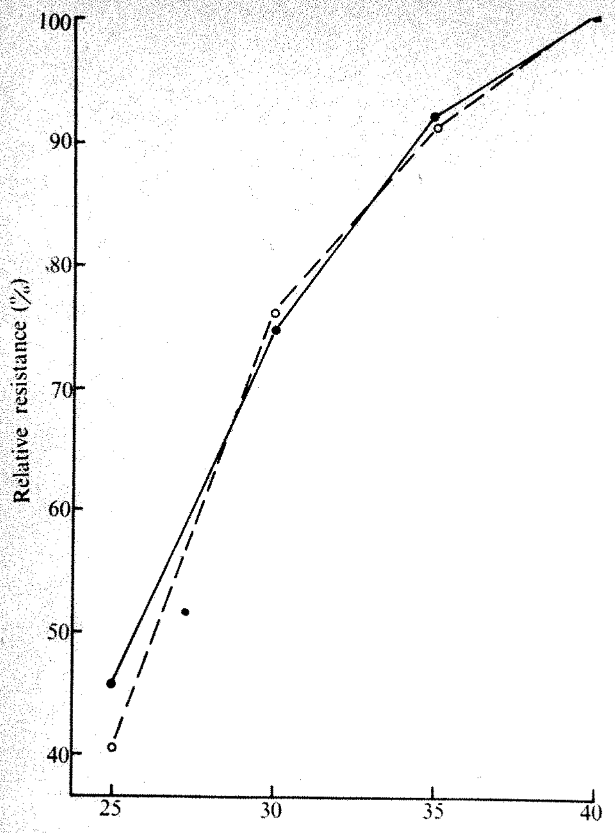


FIG. 3 Temperature dependence of reconstitution with m16 RNA and p16 RNA. Reconstitution was measured by the relative resistance to digestion by pancreatic RNase of reconstituted particles. Reactions[6] were done with 0.72 A_{260} units of a mixture of m16 (^{3}H-labelled) and p16 (^{32}P-labelled) RNA, and 44 μg total 30S proteins (61 μg per A_{260} unit). The reactions were incubated at 25° C, 30°C, 35° C, and 40° C, respectively, for 20 min in a volume of 0.5 ml. A control reaction with RNA alone was incubated at 40 C for 20° min. After the incubation, duplicate 0.2 ml aliquots of each reaction were diluted forty-five-fold in 8.8 ml of buffer composed of 5 mM Tris-HCl, *p*H 7.8, 5 mM $MgCl_2$ and 50mM NaCl at 25° C. After withdrawing duplicate 0 time aliquots of 2 ml from each reaction, 1 μg of pancreatic RNase was added and the incubation continued for 50 min at 25° C. After 50 min of incubation in the presence of RNase, duplicate aliquots of 2 ml were withdrawn. The zero time and 50 min aliquots were precipitated with 2 ml of 10% TCA containing 1 mg ml^{-1} bovine serum albumin at 0° C. After 20 min at 0° C, the precipitates were collected on glass fibre filters and washed with 40 ml of 5% TCA. The precipitable radioactivity was determined for each aliquot and the mean RNase resistance for each reconstitution reaction temperature (mean c.p.m. of four aliquots incubated with RNase for 50 min) was determined relative to the zero time measurement. The change in resistance for each reaction condition is the resistance measured minus the resistance of RNA reacted in the absence of proteins with the resistance for the reaction at 59° C taken as 100% (actual change in resistance was 48.2% for m16 and 24.5% for p16). The other reaction temperatures were plotted relative to this maximum. ●—●, m16; ○—○, p16.

to its preferential protein binding during assembly cannot be determined as yet. The preferential assembly of m16 RNA to a 30S ribosome could however be due to the cooperative nature of assembly[11,12]. The precursor RNA could be present as a population of random conformations (never having been assembled to a 30S ribosome as has m16 RNA) of which only a small proportion are in a conformation suitable for the binding of early proteins, and subsequently the late binding proteins which are required for structural assembly of a 30S ribosome. It has been shown that ribosomal proteins do effect the conformation of ribosomal RNA[13]. If proteins are present in excess, then there is a rapid shift away from the equilibrium condition among the random conformations towards the specific conformation which is required for the initiation of assembly (rapid because the assembly rates of m16 RNA are apparently similar, Fig. 3). Because a precursor form of RNA is found in immature RNP particles, themselves precursors to ribosomes, *in vivo* assembly is likely to couple protein binding, RNA methylations and oligonucleotide cleavage of p16 in a cooperative and sequential manner. A study of such interactions may be possible using p16 RNAs with varying degrees of methylation, and by carrying out the simultaneous nuclease trimming of the RNA.

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Dual Role of Gene D5 in the Development of Bacteriophage T5

THREE distinct classes of phage-specific proteins—"pre-early", "early" and "late"—are synthesised in a temporal sequence after infection of *Escherichia coli* with bacteriophage T5[1] (Fig. 2*a*). The synthesis of pre-early proteins begins within the first minute after infection and is shut off 8–10 min later[1,2]. The beginning of synthesis of early proteins is delayed until about 6 min after infection. This delay is mechanical, and is a result of the transfer of phage DNA to the host cell in two steps[1,3]. The rate of synthesis of early proteins decreases considerably after 20 to 25 min of infection, but synthesis does continue through the late period at diminishing rates. We have observed that the rate of synthesis of most early proteins is diminished normally even when the turn-on of synthesis of late proteins is defective[4]. This observation suggested to us the presence of a negative control mechanism for the 'shut-off' of synthesis of early proteins, rather than a passive shut-off, such as a competition between early and late transcripts for the translational

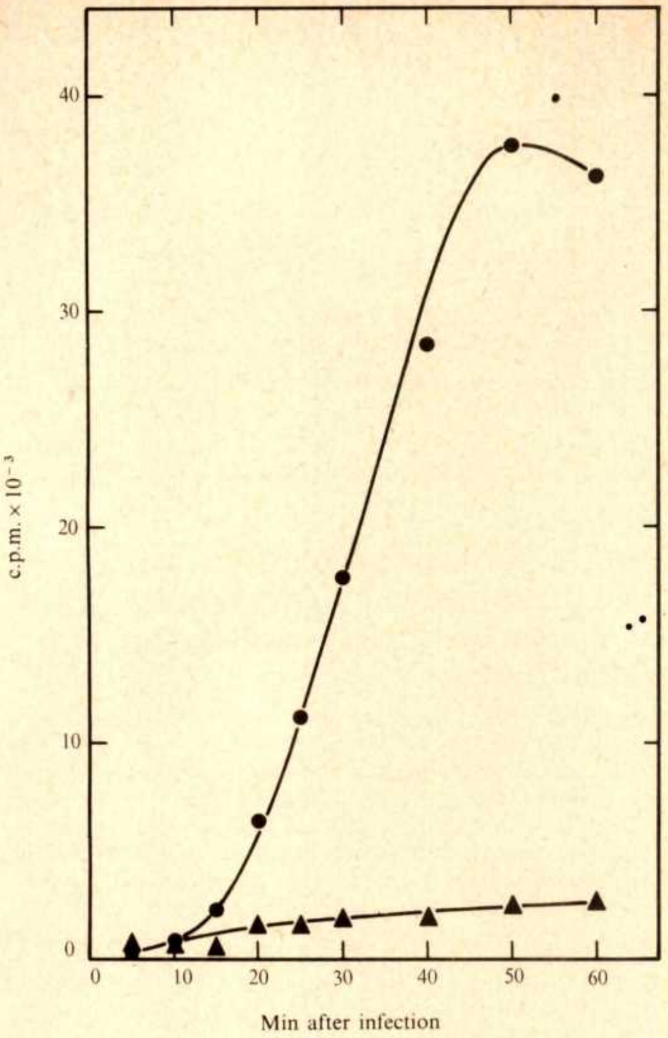

Fig. 1 Cumulative incorporation of ^{3}H-thymidine into DNA after infection of *E. coli* F (su$^-$) cells with T5$^+$ (●) and with T5(D5)*am*H12a (▲). Bacteria were grown, concentrated and infected as described in ref. 5. The phage–bacterium complexes were diluted in a maleate-glucose-salts medium containing 10^{-3} M CaCl$_2$, 250 μg of deoxyadenosine ml^{-1}, 5 μg of uracil ml^{-1}, and 2 μg of ^{3}H-thymidine ml^{-1} (specific activity, 1.75 μCi μg^{-1}). Aeration was begun immediately after dilution ('zero time') and 1.0-ml samples were taken at various times and added to 1.0 ml of ice-cold 15% trichloroacetic acid. 0.03 ml of 0.5% serum albumin and 0.05 ml of 0.25% salmon sperm DNA were added as coprecipitants. After at least 30 min at 0° C, the acid-insoluble material was collected on glass fibre filters (Reeve Angel, Grade 984 H), and washed successively with 10 ml of 5% trichloroacetic acid, 10 ml of 0.5% trichloroacetic acid and 10 ml of acetone. The filter pads were dried and counted in a liquid scintillation counter using a toluene-based scintillation fluid.

system of the infected cell. We have identified a gene the product of which is required for the shut-off of synthesis of phage specific early proteins, but which is also required for the replication of phage DNA.

Figure 1 shows that phage DNA is not synthesised in non-permissive (su$^-$) cells infected with an amber mutant defective in gene D5, whereas phage DNA synthesis begins at about 10 min after infection and continues for 40 min in cells infected with wild-type phage. Figures 2 and 3 show that the synthesis of all early proteins the synthesis of which are shut off by about 25 min after infection with wild-type phage, is not shut off after infection with a mutant defective in gene D5, with the exception of polypeptide E13. The rate of synthesis of E13 is nevertheless still appreciable 60 min after infection, although it is less than at 13 min after infection. The synthesis of early

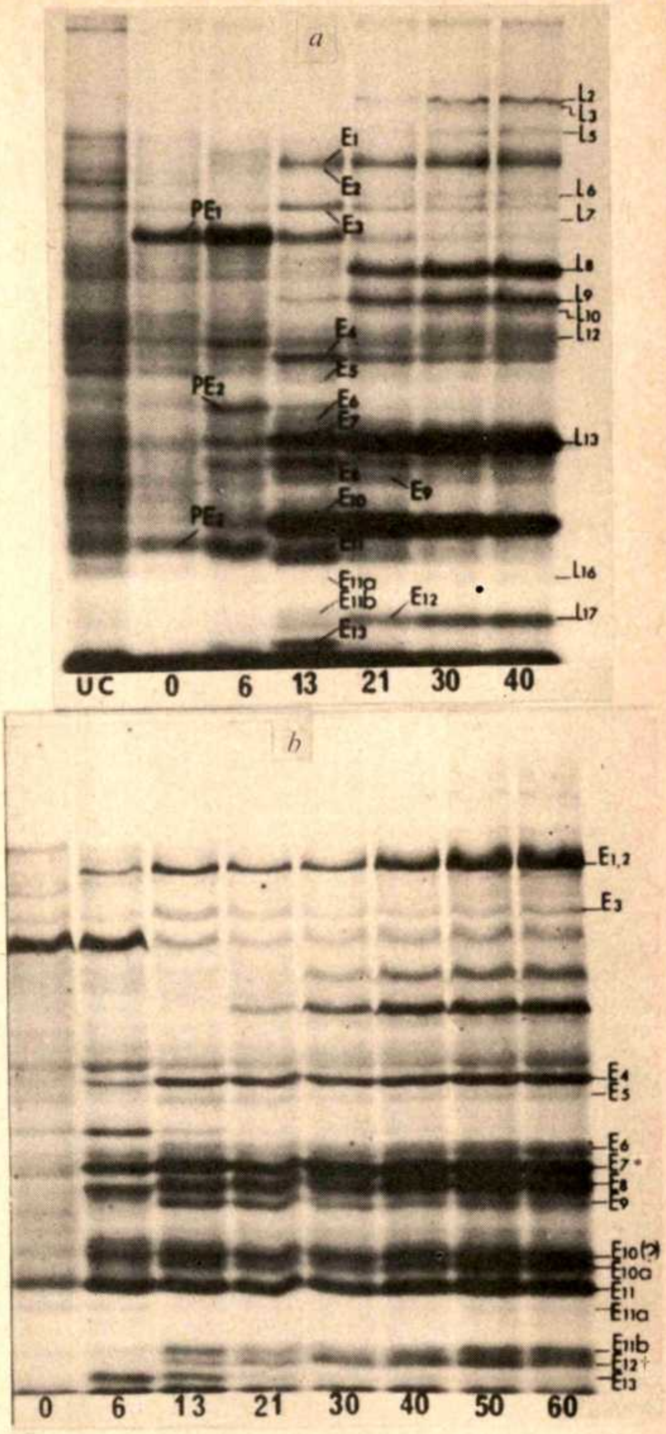

Fig. 2 Autoradiograms of electrophoretically separated ^{14}C labelled polypeptides from *E. coli* F (su$^-$) infected with T5. The electrophoresis was performed in 10% polyacrylamide gels containing sodium dodecyl sulphate (SDS). *a*, T5$^+$; *b*, T5(D5)*am*H12a. *E. coli* F cells were grown and infected as described in ref. 5. Samples of phage-infected cultures were pulse-labelled essentially as described in ref. 1 with ^{14}C-reconstituted protein hydrolysate (0.2 μCi ml^{-1}). Electrophoresis and autoradiography were carried out as described in refs 4 and 5. The numbers below the autoradiograms indicate the times when 4-min pulses were begun. The designation UC, PE, E, and L represent uninfected cells, pre-early, early, and late polypeptides, respectively. For details concerning the pre-early and late polypeptides see refs 6 and 5, respectively. * Polypeptides E7 and L13 run together in 10% gels, but can be resolved in 15% gels. Analysis of pulse-labelled polypeptides in 15% gels revealed that the synthesis of polypeptide E7 is shut off by about 25 min after infection with T5$^+$, whereas it is not shut off by 60 min after infection with T5(D5)*am*H12a. † In cells infected with T5(D5) *am*H12a, L17 is absent due to a defect in the cleavage of a head protein. Polypeptide E10 (?) in (*b*) is probably a polypeptide that has an electrophoretic mobility very similar to polypeptide E10.

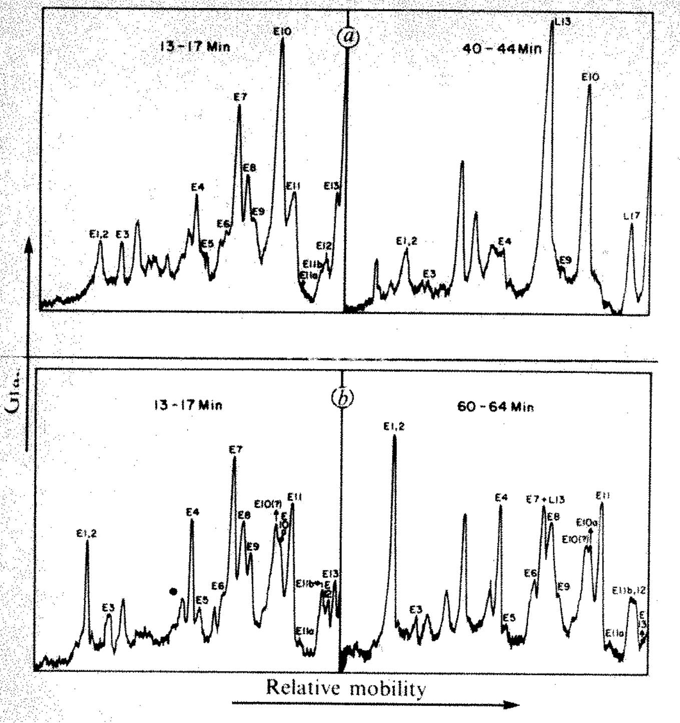

Fig. 3 Microdensitometer scans of the autoradiograms of electrophoretically separated ^{14}C-proteins from su$^-$ cells infected with T5$^+$ (*a*) and T5(D5)*am*H12a (*b*) (see Fig. 2). The designation E identifies early polypeptides. Scans were performed with a Joyce-Loebl microdensitometer.

proteins E1, E2 and E10 is not shut off at late times even in infections with wild type phage (Fig. 2*a*). Apparently, the synthesis of most early proteins is shut off by a mechanism involving the product of gene D5, but the synthesis of at least one early protein (E13) is shut off partly by another mechanism. Other DO (no phage DNA synthesis) mutants of T5 (genes D7, D8, and D9[7,8]) show normal shut-off of the synthesis of most early proteins, although one or two proteins do show slightly extended synthesis (our unpublished data).

The product of gene D5 is most likely polypeptide E10. It is synthesised in such large amounts that it masks two or three other polypeptides that have electrophoretic mobilities very close to it (Fig. 2*a*). When E10 is not synthesised, these other two or three polypeptides can be seen (Fig. 2*b*). Our preliminary results indicate that polypeptide E10 is a DNA-binding protein. Its role in the replication of phage DNA may be similar to that of other phage-induced DNA-binding proteins[9-12]. Its effect on the synthesis of early proteins may be at the level of transcription where it could act as a repressor for the synthesis of early mRNA. We are investigating the effect of the product of gene D5 on the transcription of early T5 genes *in vivo* and *in vitro*.

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On the Evidence for Partial Gene Duplication from Amino Acid Sequence of Bovine Glutamate Dehydrogenase

Bovine glutamate dehydrogenase consists of a single polypeptide chain of 500 residues[1]. Engel[2] has proposed that the regulatory site of the enzyme was produced by duplication of the amino acid sequence of the active centre, because when sections 114–163 and 269–318 are aligned there are twelve positions where identical amino acids occur in the two sections. Engel calculated that the probability of observing twelve identical positions in two random sequences of length fifty residues was 4.3×10^{-6}. He argued that as there are 352 possible sequences of fifty residues with which section 114–163 may be aligned without overlapping itself the overall probability is $352 \times 4.3 \times 10^{-6}$ ($= 1.5 \times 10^{-3}$). On this basis the observation quoted above is highly significant. We will show here that this probability is not significant.

Using a computer twenty random sequences of length 500 residues were constructed. Ten of these random sequences were drawn from an infinitely large amino acid pool with the same composition as the enzyme. Another ten sequences were constructed by 'random rearrangement' of the amino acid sequence of glutamate dehydrogenase. The compositions of the latter group of random sequences were, therefore, identical with that of the enzyme whereas in the former group the random sequences did not possess precisely the same composition as the enzyme. Taking the actual amino acid sequence of bovine glutamate dehydrogenase and the twenty random sequences we have aligned all possible pairs of sequences of length fifty residues. For each 500 residue sequence there were 101,475 alignments. The number of identical positions was counted for each alignment (Table 1). For the two groups of random sequences Table 1 shows means and standard deviations. In all cases except one the observed frequencies for glutamate dehydrogenase lie within two standard deviations of the means of the frequencies for the random sequences.

Therefore, by this test, Engel's finding of twelve identical positions in two runs of fifty residues is not significant and we conclude that there is no evidence for duplication in the amino acid sequence of glutamate dehydrogenase.

It remains possible that different ways of comparing sections of the sequences, for example by allowing the use of a small number of gaps to increase the number of identical positions or by using the theoretical minimum number of nucleotide base changes might yield a different conclusion. The use of these methods, however, also involves uncertainties and difficulties in interpretation as has been pointed out by Haber and Koshland[3] and by Dickerson[4].

Several authors have pointed out that even random sequences contain unique, but unpredictable, regularities[5]. Are sections 114–163 and 269–318 therefore the parts of the sequence which Engel's theory would predict to be homologous? There is no current evidence that section 269–318 constitutes the regulatory site and for the active centre the only definite fact is that lysine 126 is essential for enzyme activity. Since there are fifty sequences of length fifty residues which include residue 126, Engel's calculated probability for the random occurrence of twelve identical positions should be 50 × 1.5 × 10^{-3} (= 7.5 × 10^{-2}) and on this basis the observation quoted is not significant. It is not however to be seriously supposed that either the active centre or the regulatory site would correspond to fifty contiguous residues.

Table 1 Frequencies of Different Numbers of Identical Positions

No. of identical matches	Observed frequencies Bovine GDH	Random sequences Rearranged	Drawn from pool
0	5,155	5,270 ± 321	4,721 ± 406
1	15,828	15,987 ± 360	14,881 ± 952
2	23,604	23,983 ± 359	23,077 ± 277
3	23,307	23,284 ± 568	23,516 ± 482
4	17,026	16,755 ± 329	17,433 ± 727
5	9,878	9,402 ± 235 *	10,149 ± 836
6	4,504	4,357 ± 231	4,829 ± 553
7	1,508	1,712 ± 146	1,918 ± 208
8	464	546 ± 69	663 ± 102
9	164	135 ± 25	123 ± 59
10	34	32 ± 23	56 ± 29
11	2	5.7 ± 7.8	11.3 ± 8.2
12	1	1.4 ± 2.9	2.1 ± 3.6
13	—	0.1 ± 0.3	1.6 ± 4.8
14	—	—	0.1 ± 0.3

* Observed frequency more than two standard deviations from mean of frequencies for random sequences.

Although no valid evidence has so far been produced in its favour it is possible that Engel's proposal as to the origin of the regulatory site of enzymes is correct and the evidence from X-ray studies may prove important in this problem.

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Dr Engel replies:

Williams and Wilkins have done the evidence less than justice in two respects: (1) Our disagreement hinges upon the choice of a basis for statistical assessment of the observed sequence match. Absolute validity can neither be attained nor defined; one can only attempt to select reasonable criteria. My original probability calculation may have been too arbitrarily exclusive but, equally, Williams's and Wilkins's computer-search is too arbitrarily all-inclusive. To ignore relevant information must bias the calculation against discovery of the truth. The evidence for the importance of lysine-126 comes not only from studies of kinetics and binding (see ref. 1), but also from sequence comparisons which show that lysine-126 is in a conserved region[2–4]. This being so, even fifty may be an overestimate of the necessary number of sequences containing lysine-126 to be compared.

(2) Even if one concedes a probability as high as 7.5% for random occurrence of the observed degree of matching for a sequence including lysine-126, this takes no account of the additional evidence from the comparison with GPDH[5]. Here there can be no question of screening the whole molecule: the alignment is already fixed on the basis of the observed homology of sequences surrounding the lysine residues in GDH and GPDH that react with PLP[6,7]. The two GDH sequences are, of course, aligned on the basis of the internal homology. The probability of the observed matching of nine positions out of sixty-four in GDH 2 and GPDH may be calculated as follows.

The match[6,7] between GDH 1 and GPDH is assumed to reflect a genuine evolutionary relationship (six identities out of sixty-four). The match[5] between GDH 1 and GDH 2 (thirteen identities out of sixty-four) is taken, for the purposes of calculation, to be a fortuitous occurrence judiciously selected. The probability, higher than for twelve out of fifty, is 0.21, based on 64 × 310 comparisons. (For such large numbers of comparisons the approximation of multiplying the probability calculated for a single comparison by the number of comparisons breaks down and the second term in the binomial expansion must be included.)

Thirteen random matches with the sixty-four residues of GDH 1 could coincide with 0, 1, 2, 3, 4, 5 or 6 of the matching positions in the GDH 1/GPDH comparison. If no positions match in all three sequences, this defines 13 + 6 = 19 positions that differ in GDH 2 and GPDH. Thus nine matches would have to be found among the remaining forty-five pairs of residues. Similarly, one congruence in all three sequences defines eighteen positions that differ in GDH 2 and GPDH, so that eight identities must be found in forty-six comparisons, and so on. The probability of finding n positions identical in all three sequences is given by

$$P_1 = \frac{{}^{58}C_{(13-n)} \times {}^{6}C_n}{{}^{64}C_{13}}$$

The probability of finding 9-n further positions matching in GDH 2 and GPDH is given by

$$P_2 = {}^{(45+n)}C_{(9-n)} \times \frac{19^{(36+2n)}}{20^{(45+n)}}$$

The overall probability of the total of nine matches that is in fact found is

$$\sum_{n=0}^{6} P_1 P_2 = 4.2 \times 10^{-3}$$

The cumulative probability, therefore, of the two observed matches with GDH 2 is $4.2 \times 10^{-3} \times 2.1 \times 10^{-1} = 8.8 \times 10^{-4}$.

To say the least this signifies a noteworthy coincidence. The availability of more sequence data should soon provide further evidence for or against the hypothesis of partial gene duplication in GDH.

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Conformational Analysis of N^6-Methyladenylyl-Uridine

THE availability of a large Fourier transform (FT) NMR system in the laboratory of one of us (G. v. B.) allowed us to obtain a series of proton magnetic resonance spectra of nucleosides and dinucleoside monophosphates superior to any hitherto reported. We shall show that the use of the FT-technique at 270 MHz can increase our understanding of the structural features and of the dynamics of the stacking (helix-forming) processes because the chemical shifts as well as the ^1H-^1H and the ^1H-^{31}P coupling constants hidden in the high-field (ribose) part of the spectrum are now in principle amenable to analysis. These parameters provide the key to unlock the secrets of the three-dimensional time-average structure of dinucleosides in solution.

We report here the first complete assignment of the ribose protons of a dinucleoside monophosphate, N^6-methyladenylyl-uridine ammonium salt (m^{6}ApU, or N^6-MeApU, Fig. 1) and we propose a model for the various conformational equilibria based on recent studies on the pseudorotation properties of the ribose ring[1,2].

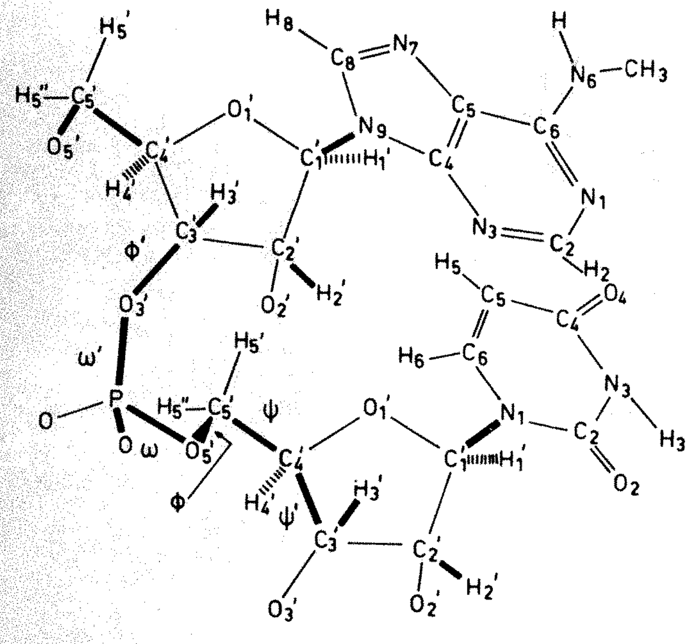

Fig. 1 Structure of N^6-methyladenylyl-uridine, showing the stereochemical relationships between the coupled nuclei as well as the principal backbone torsion angles[11].

This work forms part of our programme of research into the biological function of modified nucleosides in tRNA and rRNA[3]. The dinucleosides were prepared by the phosphotriester approach[4-5], which enabled us to prepare the desired compounds on a large scale (up to 1 g) in a relatively short time. Details of the synthesis and purification of ApU, m^{6}ApU, m$^6_2$ApU, i^{6}ApU, UpA, Upm^{6}A, Upm$^6_2$A, and Upi^{6}A will be published later. (We follow the nomenclature recommended by IUPAC-IUB[6].)

The 270 MHz ^1H NMR spectra were recorded on a Bruker HDX-270 spectrometer, using D_2O lock, in FT mode. Interferograms were stored in 16K of computer memory and chemical shifts were measured on plots varying in scale from 60.38 Hz cm^{-1} to 7.55 Hz cm^{-1}, to an accuracy of ±0.005 parts per million (p.p.m.). The shifts are measured relative to the methyl peak of a trace of 2,2-dimethyl-2-silapentane-sulphonic acid sodium salt (DSS) in D_2O, added as an internal reference. Owing to the relatively large line width (3.8 Hz) the coupling constants are accurate to only about ±0.3 Hz. The spectrum of m^{6}ApU was taken at 20° C, 0.05 M solution in D_2O. Further technical details will be published elsewhere (G. v. B. *et al.*, in preparation). Spin-spin ^1H decoupling experiments were carried out in the CW mode at 270 MHz. In addition, ^1H 100 MHz FT NMR spectra were recorded in our laboratories on a Varian HA-100 spectrometer at 4° C and at 30° C.

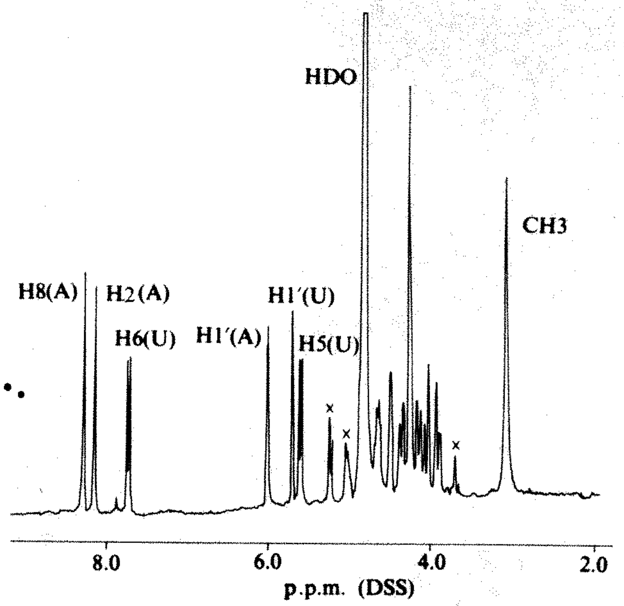

Fig. 2 270 MHz FT proton magnetic resonance spectrum at 20° C of 0.05 M solution of m^{6}ApU. Crosses indicate resonances due to unidentified degradation products.

Figure 2 shows the entire ^1H spectrum including the straightforward assignment of the peaks due to the base protons and those due to the H1′ protons. This low-field part of the spectrum is entirely analogous to that found in ApU[7], in uridine[8] (pU part) and in dimethyladenosine (C. A. and H. P. M. de Leeuw, unpublished) (m^{6}A part). The tightly coupled ribose spectrum, consisting of ten proton signals (H2′, H3′, H4′, H5′, H5″ for each residue), spans a region of less than 1 p.p.m. and has hitherto defied all attempts at complete analysis. Even FT spectroscopy at 100 MHz proved ineffective. Indeed, it was recently stated[9] that ^{13}C NMR is of considerably more use in the case of polynucleotides, where the proton NMR spectra may well be totally unanalysable to yield ^1H-^1H and ^1H-^{31}P coupling constants[9]. However, at 270 MHz the lines are well enough resolved to warrant a closer study. Figure 3*a* shows the final assignment of the observed transitions to the individual ribose protons. The decoupling experiments proved indispensable for this analysis, which is based on the following observations.

First, the two doublets at 6.02 p.p.m. and at 5.71 p.p.m. obviously belong to the H1′ protons in the m^{6}Ap and in the pU residues respectively, because in all dinucleoside monophosphates that contain adenosine and another base the H1′ (A) signal invariably appears at the lower field position. In ApU the shift difference H1′(A)-H1′(U) equals 0.29 p.p.m.[7], the corresponding difference in m^{6}ApU amounts to 0.31 p.p.m. Now irradiation (decoupling) at 4.82 p.p.m., that is, in the region of the HDO signal, caused both the H1′(A) signal as well as the peak at 4.65 p.p.m. to simplify. This observation is positive evidence for the location of two more protons belonging to the m^{6}Ap residue: H2′(A), obscured by the large HDO peak, and H3′(A) at 4.65 p.p.m. (Fig. 3). The latter signal shows a characteristic broadening (also found in the corresponding spectra of ApU and m$^6_2$ApU examined by us) which we ascribe to the stereospecific $^3J_{H\text{-}^{31}P}$ coupling, superimposed on the usual $J_{2'3'}$ and $J_{3'4'}$ pattern seen in mononucleosides.

Irradiation of H3′(A) caused changes in the line shape of the neighbouring peak occurring at 4.49 p.p.m., but, owing to the small (44 Hz) shift difference between the position irradiated and the peak observed, this result is not considered entirely conclusive. Irradiation at 4.49 p.p.m. (marked H4′(A) in Fig. 3), however, definitely caused a collapse of the fine splitting pattern of the high-field signals at 4.05 and 3.92 p.p.m.; irradiation at these latter positions in turn sharpened the 4.49 p.p.m. transition, thus providing a double check. The characteristic AB type quartet (4.05/3.92 p.p.m.) is easily identified as due to the H5′ and H5″ protons of the m^{6}Ap side chain. They appear within 0.06 p.p.m. from the corresponding H5′,5″ quartet in dimethyladenosine (C. A. and H. P. M. de Leeuw, unpublished). Therefore, the signal of the H4′(A) proton, coupled to H3′,H5′ and H5″, is identified with the 4.49 p.p.m. resonance. This completes the assignment of all ribose protons contained in the m^{6}Ap residue.

The pU protons could be assigned as follows: irradiation of the large peak at 4.26 p.p.m. caused the fine splitting of the H1′(U) signal to disappear, hence the H2′(U) proton signal is localised at or near 4.26 p.p.m. The intensity of this peak, however, corresponds to three protons, and we conclude that H3′(U) and H4′(U) accidentally resonate at approximately the same frequency as H2′(U). There remain two characteristic doublets, forming an AB quartet, flanking the 4.26 p.p.m. peak. These doublets are assigned to H5′(U) and H5″(U), shifted downfield (by 0.25–0.44 p.p.m.) with respect to the corresponding signals in uridine because of the attachment of the 5′-phosphate ester group.

Next, estimates of the chemical shifts and of a series of (first-order) vicinal coupling constants were obtained directly from the observed spectrum. Educated guesses for the few remaining ′H-′H coupling constants ($J_{2'3'}$,m^{6}Ap residue; $J_{2'3'}$,$J_{3'4'}$; pU residue) were made based on the pseudorotation model of the ribose ring[2]; determination of the ^{1}H-^{31}P coupling constants was aided by analysis of the line widths in conjunction with recently recorded values for these couplings at the 5′-position ($J_{5'P} = J_{5''P} = 4.5$ Hz in 5′-adenosine monophosphate[10]) and in the 3′-position ($J_{3'-P} = 8.0$ Hz in 3′-uridine monophosphate[9]) respectively. Unfortunately, such data on the 'reverse' monomers 3′-AMP and 5′-UMP seem to be lacking.

The computer program LAME was then used to simulate and plot the 270 MHz ribose spectra, assumed to consist of two independent seven-spin systems, to yield selfconsistent parameters, intensities, and line shapes matching those observed (Fig. 3*b*). The final parameters are given in Table 1. The relatively large line widths preclude the observation of any small long range ^{1}H-^{1}H or ^{1}H-^{31}P coupling so all these coupling constants were assumed to be zero.

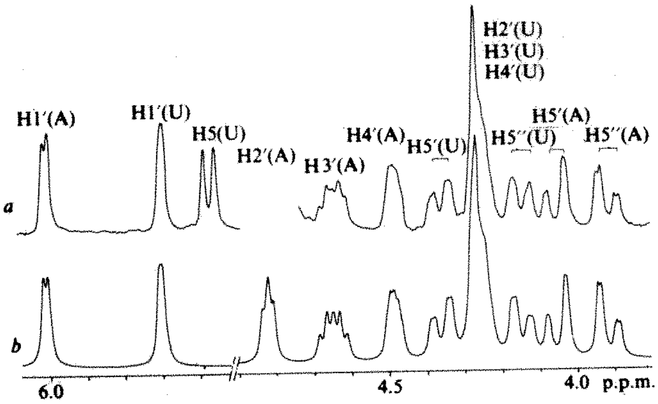

Fig. 3 *a*, parts of the spectrum of Fig. 2, eight times expanded. *b*, Computer simulated plot of the top spectrum. The H2′ signal (far left) coincides with the HDO peak in the observed spectrum.

Table 1 Chemical Shifts and Coupling Constants of m^{6}ApU, 0.05 M Solution in D_2O at 20° C*

Residue	Proton designation	Chemical shift (p.p.m.) †	Coupling constants (Hz) ‡	
N^6-mAp	H-1′	6.01	$J_{1'2'}$	3.6
	H-2′	(4.82)§	$J_{2'3'}$	5.0
	H-3′	4.65	$J_{3'4'}$	5.7
	H-4′	4.49	$J_{4'5'}$	2.3
	H-5′	4.05	$J_{4'5''}$	3.0
	H-5″	3.92	$J_{5'5''}$	−13.1
	H-2	8.14		
	H-8	8.28	$J_{3'31P}$	9.5
	CH_3	3.07		
pU	H-1′	5.70	$J_{1'2'}$	3.0
	H-2′	4.24‖	$J_{2'3'}$	5.0‖
	H-3′	4.27‖	$J_{3'4'}$	6.2‖
	H-4′	4.28‖	$J_{4'5'}$	1.9
	H-5′	4.36	$J_{4'5''}$	2.0
	H-5″	4.15	$J_{5'5''}$	−11.9
	H-5	5.60	J_{56}	8.3
	H-6	7.73		
			$J_{5'-31P}$	3.5
			$J_{5''-31P}$	3.5

* Data from 270 MHz Fourier transform spectra.

† The chemical shifts are measured relative to the methyl peak of DSS; the accuracy is of the order of 0.005 p.p.m.

‡ The coupling constants are accurate to about 0.3 Hz.

§ Estimated value. This transition is hidden under the HDO peak but irradiation at the position shown caused the H-1′ (mA) doublet to collapse to a single peak.

‖ Assumed values. Extensive overlap of the H-2′, H-3′ and H-4′ transitions of the pU moiety precludes exact spectral analysis of these signals.

The accidental coalescence of the H2′(U), H3′(U), and H4′(U) signals in m^{6}ApU proved to be a blessing in disguise. First, this feature allowed an unambiguous assignment of this ribose spectrum. Second, once this problem was unravelled, we were able to make a successful attack (unpublished) at solving the more complex patterns exhibited by the ribose spectra of ApU and of the dimethylated analogue m$^6_2$ApU.

Figure 1 depicts the m^{6}ApU molecule. Six torsional degrees of freedom, here denoted (ref. 11) ψ′, ψ, φ, ω, ω′ and φ′, determine the exact folding of the sugar-phosphate backbone. Recent X-ray studies have disclosed fine details of this backbone folding in (base–base) stacked forms of ApU[12] and of GpC[13] as they occur in the crystalline state. It is now of great interest to note that four backbone torsion angles can in principle be studied by NMR spectroscopy of dinucleoside phosphates in solution because vicinal coupling constants J_{HH}, $J_{\mathrm{H}31\mathrm{P}}$ and $J_{\mathrm{H}13\mathrm{C}}$ are torsion angle dependent. Unfortunately, the ω and ω′ angles are not directly accessible from couplings without great experimental effort (for example, by measurement of ^{13}C–^{17}O couplings.)

We believe that the present NMR results are compatible with the X-ray structures of ApU and GpC with a few qualifications.

The coupling constant J_{HX} is related to the torsion angle τ_{HX} by the Karplus equation[14,15], which we write[2]:

$$J_{HX} = A_{HX}\cos^2\tau - B_{HX}\cos\tau \qquad (1)$$

From an analysis of X-ray and NMR data on nucleosides and nucleoside phosphates recently, best values for A_{HH} (10.5 Hz) and for B_{HH} (1.2 Hz) were extracted[2]. Blackburn *et al.*[16] proposed values for A_{HP} and B_{HP} of 16.3 Hz and 4.6 Hz respectively. Some care should be exercised, because in cases where mixtures of conformations (under dynamic equilibrium conditions) are present, one may in fact measure a time-average

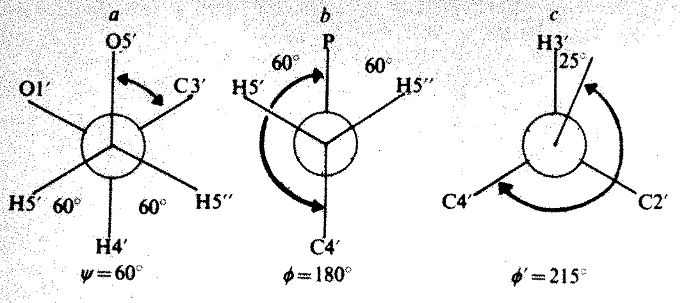

Fig. 4 *a*, Favoured rotamer about the C4′–C5′ bond ($\psi=60°$); *b*, favoured rotamer about the C5′–O5′ bond ($\phi=180°$); *c*, the favoured rotamer about the C3′–O3′ bond ($\phi'=215°$). Backbone angles are indicated by arrows.

coupling that is related to the couplings of the individual conformers by:

$$J_{av}=J_{I}X_{I}+J_{II}X_{II} \tag{2}$$

Where X represents the mol fractions of each of the conformers present ($\Sigma X_{i}=1$).

Thus, J_{av} is not directly related to the torsion angles of the individual forms. Keeping this limitation in mind (it plays a role in the interpretation of the couplings between the ribose protons), we see that the backbone torsion angles can be monitored as follows: ψ' by $J_{3'4'}$(U), ψ by $J_{4'5'}$ and $J_{4'5''}$(U), ϕ by $J_{5'P}$ and $J_{5''P}$(U), ϕ' by $J_{3'P}$(A) (Table 2). The torsion angles are defined[11] with respect to C, O or P atoms and not with respect to the hydrogens, but Newman projections of the classical rotamers will clarify the relationships (Fig. 4).

The two couplings related to ψ are small and practically equal; this is also true for the ^{1}H–^{31}P couplings related to ϕ. We feel safe to conclude that in each case a single rotamer predominates to an overwhelming extent. According to an equation proposed by Wood *et al.*[17] the value of the sum $J_{4'5'}+J_{4'5''}=$ 3.9 Hz would correspond to a conformational purity of about 90% in favour of the *gg* ($\psi=60°$) rotamer. The Karplus parameters A_{HH} and B_{HH} derived by Altona and Sundaralingam[2] require a purity even closer to 100%. The sum $J_{5'P}+J_{5''P}=7$ Hz would correspond[17] to a conformational purity of the $\phi=180°$ rotamer in excess of 90%. In fact, the present results extend, and at the same time slightly revise, the Σ/Σ' correlation line proposed by Wood *et al.*[17].

The $J_{3'P}$ value could in theory correspond to four values of τ_{HH}: $\pm 25°$ and $\pm 130°$. The latter values are disregarded, because the corresponding ϕ' angles are clearly in a 'forbidden' region[14]. Our value of $\phi'=215°$ agrees surprisingly well with the X-ray results on ApU[12] and GpC[13] (average $\phi'=212°$). The second possibility, $\phi'=265°$, has been observed to occur[11] in cytidine 3′-phosphate (orthorhombic) and cannot be excluded unambiguously from consideration. However, for the time being we prefer to postulate the existence of a single predominant rotamer about the C3′–O3′ bond characterised by $\phi'=210°$–$220°$. This interpretation is consistent with the available X-ray studies on 3′,5′-dinucleoside phosphates.

When we consider the ribose proton couplings, $J_{1'2'}$ and $J_{3'4'}$ of both residues in particular, a different situation emerges. At this point we wish to stress that the ribose couplings in m^{6}ApU show perfectly 'normal' behaviour with respect to $J_{2'3'}$ and to the sum $J_{1'2'}+J_{3'4'}$ as compared with purine and pyrimidine nucleosides where the base is known to occur predominantly in *anti* conformation[2]. According to the proposed[2] multistate dynamic conformational equilibrium model of 3′,5′-dinucleoside phosphates, the couplings $J_{1'2'}$ (as well as $J_{3'4'}$) are linearly related to the amount of stacked form present in the equilibrium and can be used (equation 2) to estimate this amount with reasonable accuracy (C. A., unpublished). Thus approximately 40% of the molecules of m^{6}ApU occur in the well defined stacked conformer at 20° C (the ribose rings occurring exclusively[2] as 3′-endo or N conformers, that is, exactly the ribose forms observed to occur in solid ApU[12] and GpC[13]). In the unstacked states the A and U ribose rings presumably do not affect each other appreciably and a normal 3′-endo/2′-endo (NS) conformational equilibrium is indicated for both residues.

Table 2 Interpretation of Observed Coupling Constants of m^{6}ApU in Terms of Torsion Angles Along the Backbone

Coupling	Hz	τ_{HH}	Corresponding backbone angle	X-ray model[13]
$J_{4'5'}$ (U)	1.9	60°	ψ 60°	55°
$J_{4'5''}$ (U)	2.0	60°		
$J_{5'P}$ (U)	3.5	60°	ϕ 180°	170°
$J_{5''P}$ (U)	3.5	60°		
$J_{3'P}$ (A)	9.5	+25°	ϕ' 265°	
		−25°	ϕ' 215°	212°

(A) and (U) denote the adenosyl and uridyl residues respectively.

Finally, combining our findings regarding the extreme conformational preference along the C4′–C5′, C5′–O5′ and O3′–C3′ bonds with the fact that both stacked and unstacked molecules are present in appreciable amounts, we conclude that these three backbone conformations under discussion do not change perceptibly on stacking or unstacking. Hence the conformation angles ψ, ϕ and ϕ' seem to have been prepared by nature in such a way that they fit both stacked (helical) and unstacked states. The same is true for the ψ' angle of the C3′-endo (N) ribose conformer. The main degrees of freedom available to these molecules on unstacking (aside from the ribose equilibria) must therefore be sought in the ω and/or the ω' angles. This hypothesis has already been put forth by several workers on the basis of known crystal structures[18,19], is in agreement with the results of all-valence molecular orbital calculations[20], and now confirmed by NMR spectroscopy in solution.

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Loss of methyl acceptor activity of ultraviolet-irradiated tRNA

THE features responsible for the recognition of specific sites for methylation by tRNA methylases are presently not understood. Studies by Kuchino and Nishimura suggest that a specific octanucleotide sequence is recognised by rat liver guanosine methylases[1]. Furthermore, reports from Nishimura's laboratory show that a large (three-quarter) fragment from *E. coli* methionine tRNA could be methylated enzymatically[2]. Shershneva *et al.*[3], however have shown that fragments are not methylated until recombined. Studies in our laboratory have demonstrated that certain fragments from a mixture of *E. coli*[4] tRNAs can serve as substrate for rat liver tRNA methylases. These fragments are believed to have an average S value of 0.64 and therefore would be about ten nucleotides long. Using ultraviolet-induced alterations, we have investigated further the importance of the molecular shape of tRNA as it affects its recognition by tRNA methylases.

E. coli tRNA was obtained from General Biochemicals, Chagrin Falls, Ohio. Aqueous solutions of *E. coli* tRNA (1 mg ml^{-1}) where irradiated at room temperature with an ultraviolet lamp (MR-4, 110 V, AC60 cycle, George W. Gates and Co. Franklin Square, Long Island) for varying times in quartz cuvettes 4 cm from the source. Control aliquots of the tRNA solutions were placed at the same temperature for the same time period. The tRNA samples were assayed by existing methods[5] for their ability to be enzymatically methylated.

E. coli tRNA (10 mg) was incubated at 0°C for 20 min in 2 ml of 10 mM Tris buffer (*p*H 7.0) which contained 1 mM iodine and 0.5% (w/v) potassium iodide. The tRNA was precipitated with two volumes of ethanol and dialysed overnight against distilled water.

Ultraviolet irradiation of *E. coli* tRNA causes a marked decrease in its ability to serve as a substrate for tRNA methylases from rat liver (Fig. 1). The protective effect of magnesium towards maintaining conformation seems to be related to this effect since there was a tendency for 10 mM concentrations of magnesium to prevent the decrease in methylation. One of the primary alterations induced by ultraviolet irradiation of *E. coli* tRNA is the formation of a heterodimer formed between the nonadjacent residues 4-thiouridine and cytidine by 335 nm or 254 nm irradiation[6]. We therefore studied ultraviolet-induced alterations in 4-thiouridine in tRNA (Fig. 2). A possible correlation between the irradiation-induced changes in 4-thiouridine and the decreased capacity of the molecules to become methylated, can be examined by the effect of iodine on the methyl acceptor ability of tRNA. Iodine is a very selective reagent and only reacts with thiouridine residues[7]. Untreated *E. coli* tRNA (10 µg) gave values of 3,369 ± 270 c.p.m. when incubated with crude rat liver enzymes and S-adenosyl-(methyl-C^{14})-L-methionine. *E. coli* tRNA preparations, which had been treated with iodine for 10 min and had lost the 340 nm absorbance peak, had similar methyl incorporations (3,449 ± 223 c.p.m. per 10 µg). Thus, the decrease in methylation due to ultraviolet irradiation of tRNA seems to be due to changes at some other locus in the tRNA molecule apart from thiouridine. The irradiation of tRNA has two major effects, one is the formation of U-U dimers and the other is the destruction of thiouridine residues. Since the loss in methyl acceptor activity could not be duplicated by iodine treatment, it is unlikely that the decrease in methyl acceptor activity is

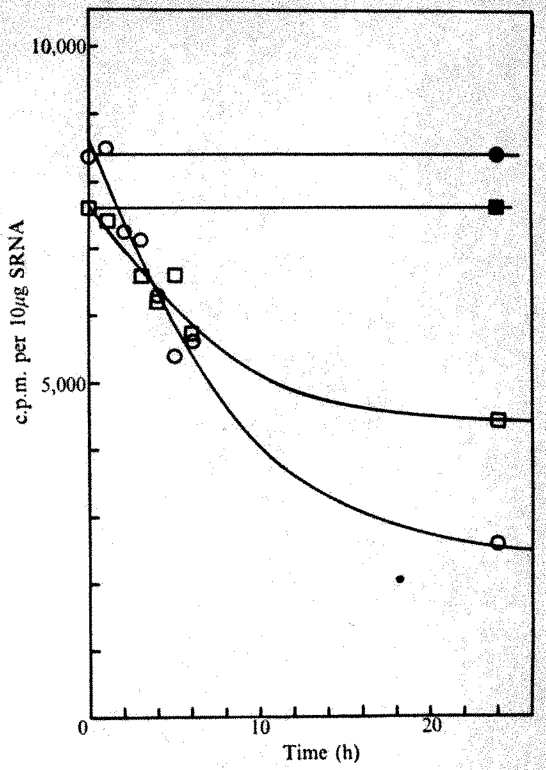

FIG. 1 Effect of ultraviolet irradiation on the methylation capacity of tRNA. Aqueous *E. coli* tRNA (3 ml, 1 mg ml^{-1}) with (□) or without (○) magnesium chloride (10 mM) were irradiated with an ultraviolet source as described in the text. The reaction mixture for the tRNA methylase assay, containing 20 µmol Tris buffer (*p*H 8.3), 10 µg aliquots of the particular tRNA preparation, 0.1 µCi of S-adenosyl-L-methionine (methyl-^{14}C) specific activity 55.2 mCi mmol^{-1}, 600 µg protein equivalents of 100,000 *g* supernatant fraction of rat liver in a total volume of 0.25 ml, was incubated for 80 min at 45° C. Unirradiated controls with (■) or without (●) magnesium were maintained at the same temperature.

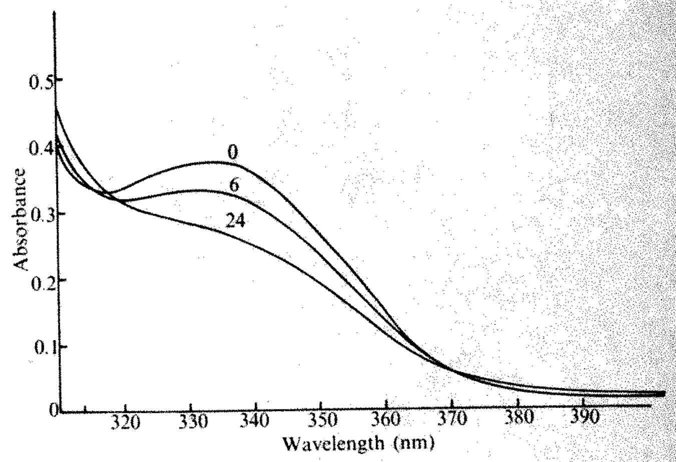

FIG. 2 Spectra of 4-thiouridine in the irradiated tRNA preparations used for methylation. Numbers on spectra refer to hours of ultraviolet irradiation as described in the text.

due to the loss of thiouridine. Thus, it is more probable that the U-U dimer is important in the loss of the activity. This agrees with a theory that tRNA methylases require a uridine residue next to the methylation site to complex with the adenosine in the S-adenosyl-L-methionine (personal communication from J. Hilderheim).

Thus ultraviolet irradiation of *E. coli* tRNA produces a marked decrease in its efficiency to serve as a substrate for tRNA methylases from rat liver. Indirect preliminary evidence suggests that U-U dimer formation is the primary molecular alteration causing this decrease.

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Ion Fluxes in Disk Membranes of Retinal Rod Outer Segments

THE process of transduction in vision is that mechanism whereby absorption of light by a photoreceptor cell in the retina results in a polarisation of the receptor cell membrane, excitation of the cell, and the eventual generation of a neural impulse to the brain from higher order neurones. It is already well known that the initial and primary process in vision is the absorption of photons by 11-cis retinal and concomitant isomerisation of the visual pigment to all-trans retinal. We have therefore studied events resulting from this photoisomerisation, events which provide a link between the absorption of light and the excitation of the receptor cell.

Yoshikami and Hagins[1] introduced a hypothesis which postulated that calcium ions were released from disk membranes of the retinal receptor cell and in turn blocked the flow of sodium ions across the plasma membrane of the cell, thereby leading to hyperpolarisation of the receptor. The implication of calcium in blocking the 'dark' current, or the movement of Na^+ across the plasma membrane in the dark adapted state of the receptor cell, concerns us in the present paper, as well as the mechanisms whereby the disk membranes' supply of Ca^{2+} is renewed, the latter process presumably relying on an active uptake mechanism of some description. We report the results of experiments which suggest that the action of light ultimately induces release of Ca^{2+} from photoreceptor disk membranes of the vertebrate rod outer segment. We report evidence as well that these disk membranes possess an active mechanism for accumulating Ca^{2+}.

If Ca^{2+} is released by the disk membrane, it must be able to replenish its supply of intradiskal calcium if the photoreceptor is to recover its cell potential and be capable of repetitive hyperpolarisation. Such recovery of Ca^{2+} must inevitably depend on an active membrane ion pump which is capable of concentrating Ca^{2+} against a gradient. Were such a pump not present, the cell potential would not be maintained and the light-induced electrical response lost.

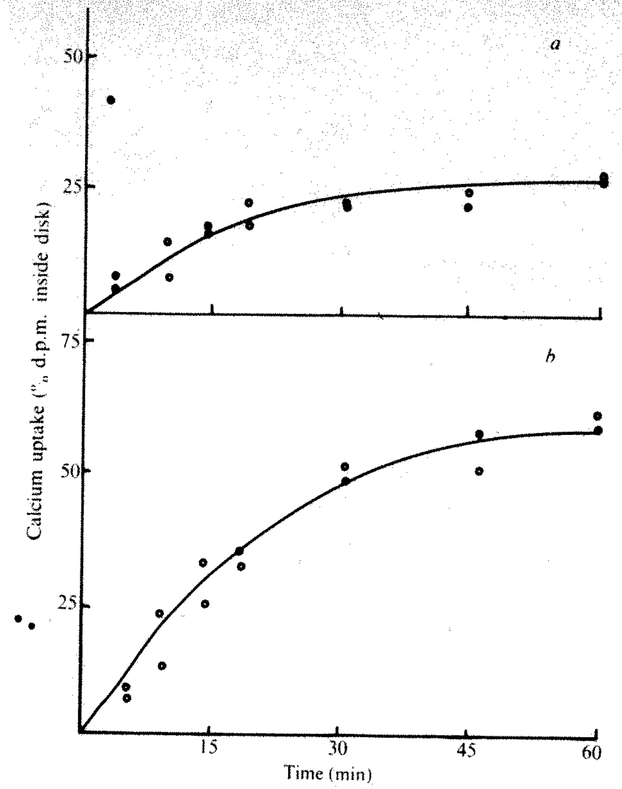

Fig. 1 Uptake of calcium by disk membranes *a*, in the dark; and *b*, in light. Membranes were incubated in 100 mM Tris buffer containing 10^{-5} M Ca^{2+} at 23° C

To investigate the presence of pump activity in the frog rod (*Rana catesbeiana*), we have developed a technique whereby specific ions or substances can be introduced into the intradisk or extradisk milieu of disk membrane vesicles. Retinae were gently dissected under red light and the rod outer segments shaken into 100 mM Tris buffer, *p*H 7.2. The crude outer segments obtained by this procedure were subsequently purified by the technique of Mason *et al.*[2]. We then used the technique reported by Mason and Lee[3] to make small vesicles of the photoreceptor membranes by sonication in an appropriate Ca^{2+}-containing media to be described below, and subsequently incubating to allow membrane resealing. By this method, rods subjected to ultrasonic disruption for 15 min in 100 mM Tris buffer resealed in approximately 45 min. As evaluated by negative staining followed by electron microscopy, this treatment results in membrane sheets which reseal tightly to form vesicles of 800 Å diameter. The sonication temperature was controlled at $2^{\circ} \pm 3^{\circ}$ C by means of a special coolant cell. No protein denaturation was apparent as indicated by the absorbance at 500 nm (A_{500}) of the visual pigment. Furthermore, negligible leakage of Ca^{2+} was observed after resealing of the vesicles, that is, about 1–2% over a period of one hour.

In our studies of ion uptake and accumulation in the photoreceptor disk preparation, we used $^{45}Ca^{2+}$ in the extradisk space and studied Ca^{2+} uptake in the dark- and light-adapted photoreceptor membrane. Preliminary experiments on an isolated, intact disk membrane preparation and buffer-washed rods apparently yield results identical to the sonicated vesicle preparation with respect to the ability of disk membranes to accumulate Ca^{2+}. Disk membranes from twenty retinae were suspended in 100 mM Tris buffer (*p*H 7.2) containing

10^{-5} M Ca^{2+} with 50 µl $^{45}Ca^{2+}$ tracer (0.3417 mCi ml^{-1}) in a reaction volume of 5.0 ml. After various times up to 1 h of incubation in both dark and light, the suspension was made 0.25 mM in EGTA and centrifuged at 50,000*g* for 30 min. The supernatant was then taken off and the pellet solubilised in 5% cetyltrimethyl ammonium bromide (CTAB). Both pellet and supernatant were placed separately in 15 ml Brays solution and counted in a Picker-Nuclear scintillation detector.

The results of these experiments are given in Fig. 1. The disk membrane seems to contain an active pump which is capable of actively accumulating Ca^{2+}. Preliminary results indicate this pump is fuelled predominantly by an ATP-Mg system. If the disk membranes were examined after 30 min of incubation at 23° C, it was found that they had concentrated Ca^{2+} against a gradient, that is they had removed nearly 50% of the total Ca^{2+} from the extradisk solution. This result is highly significant because the disk membranes occupied at most 5% of the total suspension volume. Of further interest is the fact that the pump seems to be more active in fully light-adapted membranes (Fig. 1*b*). Bleached membranes incubated in the dark with $^{45}Ca^{2+}$ accumulate Ca^{2+} at a rate nearly three times that of dark-adapted membranes.

Ion fluxes in the vertebrate photoreceptor are difficult to study, particularly at the disk membrane level. The primary reason for this is the existence of a plasma membrane which surrounds the saccules, and this membrane is apparently capable of concealing intracellular ionic changes. Using a preparation of sonicated vesicles similar to that described above, we have examined light-induced fluxes of Ca^{2+} from the inside to the outside of disk fragments. We placed $^{45}Ca^{2+}$ inside disk membrane vesicles by sonication in 50 µl $^{45}Ca^{2+}$ and 10^{-4} M Ca^{2+} in 100 mM Tris and resealing. After 2 h, the preparation was exposed to light at 23° C in the presence of a 100 mM Tris buffer containing 0.25 mM EGTA for intervals ranging from 0–120 s. Addition of EGTA in this manner prevented pumping of Ca^{2+} back into the vesicles. At this point the membrane suspension was centrifuged as before, the supernatant removed, and the pellet dissolved in 5% CTAB. The samples were counted and corrected from counts to disintegrations min^{-1}. Before counting, the absorption spectrum of the solubilised pellet was taken on a Cary 14 spectrophotometer. By this technique, it was determined that after 2 min the visual pigment was completely bleached from native rhodopsin to metarhodopsin$_{380}$ II.

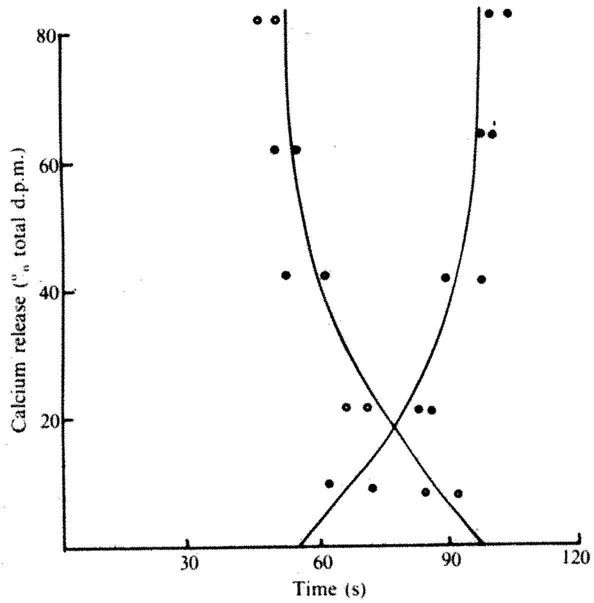

Fig. 2 Light-induced calcium release by sonicated bovine photoreceptor membranes over a time course of 120 s bleaching. Filled circles indicate percentage of Ca^{2+} in the extradisk space and open circles the percentage of Ca^{2+} in the intradisk space.

This experiment revealed that disk membranes release a large increment of Ca^{2+} upon light exposure (Fig. 2). Furthermore, these experiments seem to demonstrate that Ca^{2+} release is stepwise in response to rhodopsin photolysis, that is, upon partial bleaching of the membrane preparation, a certain amount of the intradisk Ca^{2+} is released, and no subsequent Ca^{2+} efflux occurs until further bleaching is effected.

We have also examined the stoichiometry of Ca^{2+} efflux relative to the extent of visual pigment bleaching. By determining the ΔA_{500} (difference in the absorbances of the partially bleached sample and the sample following full bleaching) of membrane samples solubilised in CTAB, one can calculate the number of mol of visual pigment bleached using an appropriate extinction coefficient (in this case, 41,000 cm^{-1}). Our initial experiments indicate that light-induced calcium efflux from the disk membrane is related to rhodopsin bleaching by a value close to unity; that is, the ratio of mol rhodopsin bleached to mol of Ca^{2+} released from a disk membrane vesicle is nearly one over a wide range of light intensities (from 5,000 photons per rod upwards to saturation and full bleaching).

The above preliminary results suggest three important properties of the disk membranes of rod outer segments: (1) they can actively accumulate Ca^{2+} in the dark and light, (2) the rate of accumulation is nearly three times greater in light than in dark, and (3) the disk membranes release Ca^{2+} as a consequence of light absorption and visual pigment bleaching. These results provide an initial experimental basis from which to pursue studies of the coupling of photon absorption and receptor cell excitation.

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Seasonal Changes in Human Plasma Levels of 25-Hydroxyvitamin D

THE relative importance of the two alternative sources of vitamin D, endogenous skin synthesis by the action of solar ultraviolet rays and dietary ingestion, is uncertain in Britain. In the past, classical rickets was common in springtime but rare in autumn[1,2], suggesting that summer sunshine was an important source of vitamin D. Seasonal variation in plasma antirachitic activity has been detected in more southerly latitudes in the United States, using bioassay techniques[3], but data from Britain are scanty and some workers have considered the effects of sunshine to be minimal in this climate[4]. Possible age or sex differences in vitamin D nutritional status are also unknown.

Before it can act, vitamin D must first be converted to its 25-hydroxy derivative (25-OH-D) in the liver[5], and this chief circulating metabolite can be measured by radio-stereo-assay, thus providing a precise index of vitamin D nutritional status[6]. Using this method, we have therefore determined plasma 25-

Table 1 Plasma 25-OH-D levels among 198 healthy white British subjects of both sexes sampled over a period of 22.5 month and grouped according to age and season

Population data			Season	Plasma 25-OH-D (ng ml^{-1})		Dietary vitamin D intake (IU d^{-1})	
Age range (yr)	Sex	Number		Mean	Range (95% confidence limits)	Mean	Range (95% confidence limits)
12 to 17	M only	59	Late September–early October 1971	22.6‡	13.4–37.9	147 (n=38)	43 to 495
7 to 10	Both	26	October–early November 1972	21.3	10.1–45.3		
18 to 37	Both	27	October–early November 1972	21.3	10.8–41.8	150 (n=19)	47 to 484
75 to 86	Both	18	Late November 1972–late January 1973	12.4*	5.1–29.8		
14 to 17	F only	15	Late January 1973	12.9*	7.2–23.2		
18 to 37	Both	19	Late March 1973	12.9*	6.6–25.2		
14 to 17	F only	14	Early July 1973	17.0†	7.6–38.4		
19 to 36	Both	20	Early August 1973	20.4	9.7–42.8		
	Total	198					

* Difference from all other mean values (except †) $P<0.001$; difference from † $P<0.05$. † Difference from ‡ $P<0.01$.

OH-D levels at different seasons over a total period of nearly 2 yr among healthy subjects of different ages. Results have shown a marked seasonal variation and we conclude that summer sunlight in Britain is a major determinant of vitamin D nutrition at the present time.

Healthy white subjects (198) of different ages and of both sexes were studied in 8 groups (Table 1). Young adults consisted of healthy students, academic and technical staff; children aged 7 to 17 were sampled from schools in the London area and they and their parents were informed volunteers; healthy geriatric subjects were statistically selected before participating in a nutritional survey in the London Borough of Camden. Plasma levels of calcium, phosphorus and alkaline phosphatase were also determined, and dietary vitamin D intakes were assessed from dietary tables in some of the school children and young adults. Plasma samples were deep-frozen before analysis for periods up to 22.5 months so that two or three populations were always compared in each assay. 25-OH-D seems to be very stable in human plasma because similar results were always obtained from replicate assays on the same specimens even after repeated freezing and thawing of the sample during the period of study. Most samples were analysed at least twice in separate assays, the coefficient of variation of replicate analysis in our hands being ±10%.

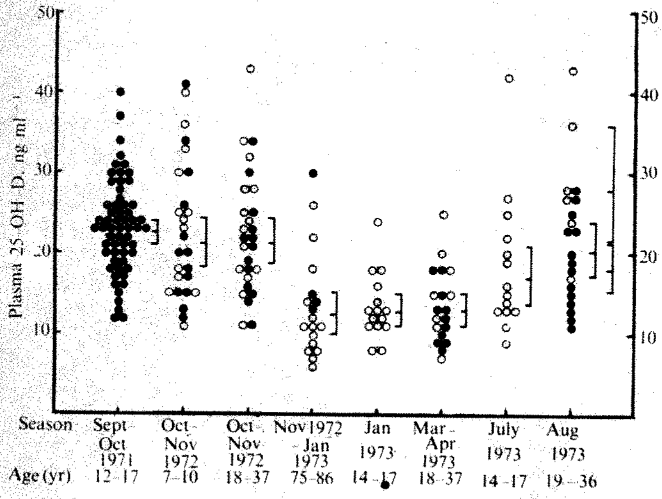

Fig. 1 Plasma 25-OH-D levels among 198 healthy white British subjects of both sexes grouped according to age and season. Bars indicate the mean and limits of 2×s.e. after log transformation and reconversion; values in August 1973 are shown for males and females both separately and together. ●, Males; ○, females.

Plasma 25-OH-D levels in the eight populations, grouped according to age and season, are shown in Fig. 1. A plot of cumulative frequencies indicated that their distribution was skewed. Mean levels after log transformation and reconversion, with the normal range (95% confidence limits), are shown in Table 1. The three populations sampled in the winter of 1972–73 and spring 1973 were similar to each other and showed much lower mean 25-OH-D levels than either autumn groups of 1971 and 1972 or the summer group of August 1973. Levels rose from March to July 1973 and approached previous autumn values by August 1973. All ages showed similar values when sampled during the same season with the possible exception of the geriatric group; because sampling in this group began in late autumn a higher mean value might have been expected. Further investigation in elderly subjects is in progress. Comparison of plasma 25-OH-D levels in boys at each year of age from 12 to 17 showed no change during puberty. Plasma alkaline phosphatase levels rise during the puberty growth spurt[7] but there was no evidence that this 'flare' is associated with relative vitamin D deficiency, and we have previously reported the lack of correlation between individual plasma levels of alkaline phosphatase and 25-OH-D[8]. A sex difference was found in adults in August 1973 when females showed significantly higher levels than males ($P<0.01$, see Fig. 1). An apparent tendency towards this sex difference at other times was not significant. Although a physiological difference between sexes in this respect cannot be excluded, a simpler reason may be that a summer dress gives greater skin exposure than does a shirt and trousers.

Dietary vitamin D intakes, in those subjects in whom it was assessed, are shown in the table. Five of the thirty-eight school boys gave intakes of less than 70 IU daily but all subjects showed normal plasma levels of calcium, phosphorus and alkaline phosphatase. No significant correlation was observed between plasma 25-OH-D and dietary vitamin D intake in either population (correlation coefficient $r=0.04$ for both groups) or between 25-OH-D and plasma calcium levels in any group (maximum correlation coefficient $r=0.228$, $t=1.765$, $0.1>P>0.05$).

Our finding of a prominent seasonal variation in plasma 25-OH-D levels indicates that contrary to some views[4], summer sunlight is an important, and possibly at present the chief, determinant of vitamin D nutrition in Britain. The skewed distribution of plasma 25-OH-D presumably arises from minority participation in outdoor activity or sunny holidays; for example, much higher summer levels of 25-OH-D have been reported in the United States among lifeguards whose exposure to sunshine is particularly high[6]. The low dietary vitamin D intakes found in some healthy subjects and the somewhat surprising absence of significant correlation between intake and plasma 25-OH-D in autumn are further evidence for the greater contribution of summer sunlight than that of diet to nutritional status. In the United States, foods are widely fortified with the synthetic compound ergocalciferol (vitamin D_2) which undergoes 25-hydroxylation in the same manner as the natural vitamin cholecalciferol (vitamin D_3); both metabolites are measured equally by the assay[9]. Haddad has recently separated both 25-hydroxy-derivatives in human plasma in the United States and found the major constituent to be 25-hydroxycholecalciferol, strongly suggesting that skin synthesis in that climate plays a more important role than dietary ingestion[10]. Although we had no reason in the present study to suppose that the actual choice of diet, with regard to vitamin D content, changes with the seasons, it is still possible that part of the seasonal variation in plasma 25-OH-D may result from a natural increase in the vitamin D content of certain foods during summer.

Although the concentrations of many plasma constituents vary with age and between the sexes, prominent seasonal variation of an important constituent has not previously been documented in tables of normal values. Present data are of clear clinical importance; rickets from whatever cause may take months or years to develop and if it is first diagnosed in autumn when early healing may be evident[11], the existence of vitamin D deficiency may only be recognised if the relatively high normal range for 25-OH-D in autumn is appreciated. Similar problems apply to the interpretation of surveys in populations at risk such as the immigrant Asian community in Britain.

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Gas Vapour Phase Constituents and SH Reactivity of Cigarette Smoke Influence Lung Cultures

Cell cultures exposed to the gas vapour phase of cigarette smoke have been reported to display essentially the same alterations as those exposed to whole cigarette smoke[1–4]. Furthermore, animals inhaling either whole smoke or the gas vapour phase of cigarette smoke have been reported to show increased pulmonary carcinogenesis[5,6] and immunosuppression[6–8].

In view of the widely accepted concept that it is predominantly the particulate matter, the 'tar', of cigarette smoke, which is responsible for lung cancer in man[9], the exploration of the biological effect of the gas vapour phase of cigarette smoke seems warranted. We report here a study designed to compare effects of two types of cigarette smoke, containing the same amount of particulate matter but differing in their content of gas vapour phase constituents, on hamster lung cultures.

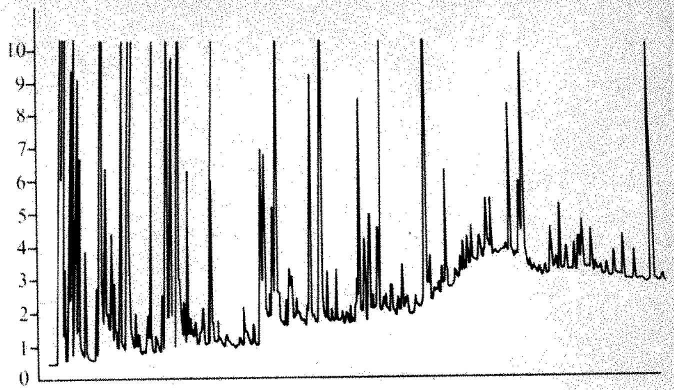

Fig. 1 Gas chromatogram of cigarette smoke without a charcoal filter.

The cigarettes were made of commercial tobacco, had a weight of 1,020 mg per cigarette, a length of 80 mm, a diameter of 8 mm, with a paper porosity of 25 ml cm^{-2} mm^{-1}, and had a white filter of cellulose acetate, length 18 mm. The lung cultures were prepared from golden hamsters 3 to 5 d old and exposed under standardised conditions in a Filtrona CFM 12 smoking machine to fresh puffs of cigarette smoke[2]. Before exposure, the cigarettes were placed in a glass container 3.5 cm long and 0.9 cm wide. To reduce the gas vapour phase content in one type of cigarette, 230 mg of activated carbon was placed in the glass holder. The activated charcoal was prepared from coconut shell, had an active surface of 1,200 m^2 g^{-1}, a CCl_4 activity of 65%, and a granulation (Tyler mesh) of 32. Special care was taken that the freshly activated charcoal was placed in the glass container immediately before each exposure, otherwise, as mentioned previously[10], the activated charcoal becomes saturated with vapour and looses its activity.

Eight different experiments were carried out, comprising nearly 3,000 hamster lung cultures. For each experiment, a minimum of twelve sets of cultures consisting of thirty-six cover-slips and twelve bottles with primary matched lung cultures was used. The sets comprised control cultures not exposed to cigarette smoke, cultures exposed to three puffs per day (25 ml at intervals of 58 s) of fresh smoke from cigarettes without a charcoal filter for 3 consecutive days, and cultures exposed in the same manner to four to six puffs from cigarettes with a charcoal filter. It was necessary to give the smaller

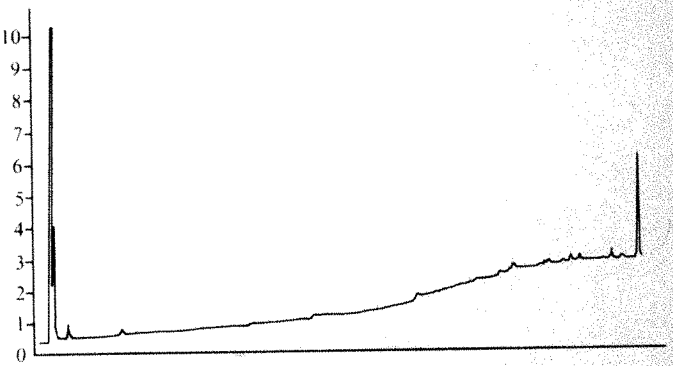

Fig. 2 Gas chromatogram of cigarette smoke with a charcoal filter.

Table 1 Comparison between the effects of fresh cigarette smoke with a high or low content of gas vapour phase and SH reacting constituents on hamster lung cultures

	Characteristics of smoke					Morphological and cytochemical alterations				
						Stage I		Stage II Abnormalities of:		
Type of cigarette	Nicotine (mg per cigarette)	Total particulate matter (mg per cigarette)	Amount of gas vapour phase	SH-index (%)	Number of exposures (puffs per exposure)	Cytotoxicity	Loss of macrophages	Mitosis $\frac{\text{Exposed}}{\text{Control}}$	Proliferation	DNA content in chromosomes (n=1,200)
Commercial tobacco	1.4	25	++++	44	3 to 20 (3)	++++	+++ to ++++	3.7±0.4 pCo 0.005	++++	++++
Commercial tobacco +charcoal filter	1.6	22	+	7	3 to 20 (4 to 6)	+*	+	1.3±0.2	+ to ++	+ to ++
None (control)	–	–	–	–	–	–		–	+	+

+ to ++, Slight to moderate; +++ to ++++, pronounced; n, Number of metaphases and telophases measured.
* Doubtful.

number of puffs from cigarettes without charcoal filters, because of their marked cytotoxic effects on the cultures. Subcultures were prepared from controls and exposed cultures once a week, and the latter were exposed again to smoke as described above. Media were changed either immediately or 5 min after exposure.

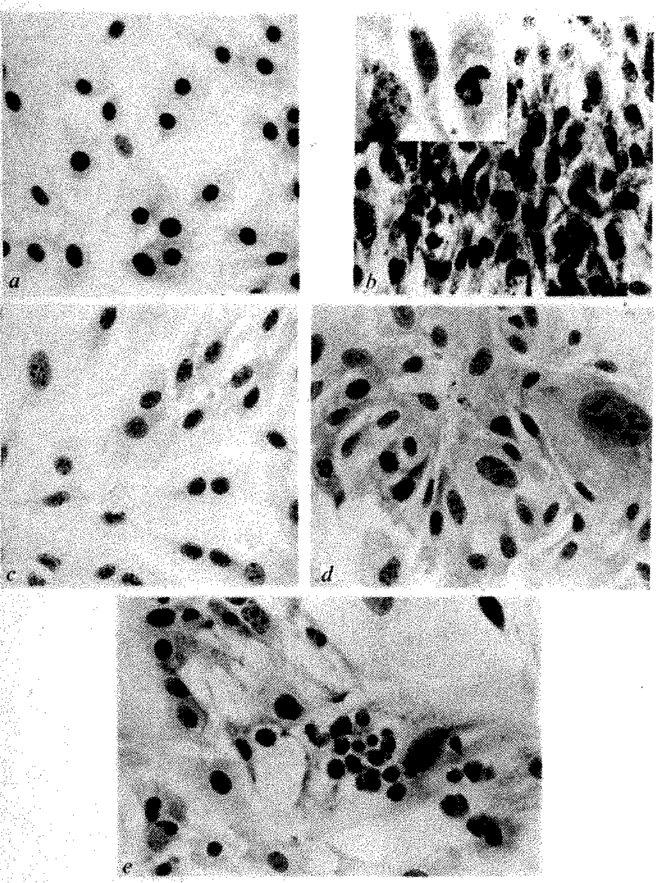

Fig. 3 *a*, Fibroblastic cells in monolayer of a 186 d control hamster lung culture stained with haematoxylin and eosin (H and E) (×178.50). Note well separated cells. *b*, The same culture as in *a*, but 26 d after sixteen exposures to three puffs each of fresh cigarette smoke without a charcoal filter. (H and E, ×178.50). Note irregularity of growth, abnormal mitosis and irregular shape of nucleus (inset ×382.50). *c*, The same culture as in *a*, but in 26 d after sixteen exposures to six puffs each of fresh cigarette smoke with a charcoal filter (H and E, ×178.50). Note similarity of culture with control culture. *d*, Fibroblastic cells of a 77 d hamster lung culture, 66 d after three exposures to three puffs each of fresh cigarette smoke without a charcoal filter (H and E, ×191.25). Note absence of macrophages, irregular sizes of nuclei and abnormal mitosis. *e*, The same culture as *d*, but 66 d after three exposures to six puffs each of fresh cigarette smoke with charcoal filter (H and E, ×191.25). Note presence of macrophages.

In view of the importance of SH groups in cell metabolism and division[11], the smoke of both types of cigarettes was examined for constituents reacting with SH groups. The SH index of the smoke was determined in the following manner: sixty cigarettes were smoked in a rotary smoking machine, according to CORESTA standards. The continuous stream of smoke was passed through a 1 l glass flask which was rotated in a water bath at 40° C. The flask contained 5 ml of a 0.1 M solution of cysteine hydrochloride in water. After the exposure to smoke, the content of the flask was transferred to a titration vessel, and the remaining cysteine which had not reacted with the smoke was titrated with copper sulphate. The results were expressed as the percentage of cysteine consumed by the smoke of sixty cigarettes (SH index).

The sequential alterations were studied in live cultures under phase contrast microscopy and in the original stained cultures from 1d to 8 months after exposure. The frequency of mitotic abnormalities was obtained by counting all abnormal mitoses and relating them to the total number of mitoses in each culture. DNA determinations were carried out by Feulgen microfluorometry[2] on the metaphases and telophases of the original stained lung cultures.

Gas chromatograms of smoke from cigarettes with a charcoal filter displayed a striking reduction of the gas vapour phase constituents and of the SH index, whereas the content of nicotine and total particulate matter closely resembled that of the smoke from cigarettes without a charcoal filter (Table 1, Figs 1 and 2). Both types of smoke produced results in all cultures, although the degree of response to cigarette smoke without a charcoal filter was not always the same for primary lung cultures prepared from different hamsters.

There were significant differences between control cultures and those exposed to smoke from cigarettes without a charcoal filter. From 1 to 4 d after exposure, marked cytotoxic effects, such as pycnosis, necrosis, cell death and a striking reduction of alveolar macrophages were observed in the cultures (Table 1). This cytotoxic effect was followed by atypical growth, a high frequency of mitotic abnormalities, and alterations in the DNA content of the chromosomes (Table 1, Figs 3*a*–*e*, 4 and 5). These atypical proliferative alterations were essentially the same as those observed in human lung cultures exposed to smoke from Kentucky Standard and Marijuana cigarettes[12,13]. Cultures exposed to smoke from cigarettes with a charcoal filter showed much milder changes of all these features, and thus resembled more closely the control cultures (Table 1 and Fig. 3*a*–*e*). Macrophages were observed in both young and older cultures (Fig. 3*e*). The differences in the DNA complement of chromosomes between control cultures and cultures exposed to cigarette smoke with and without a charcoal filter are

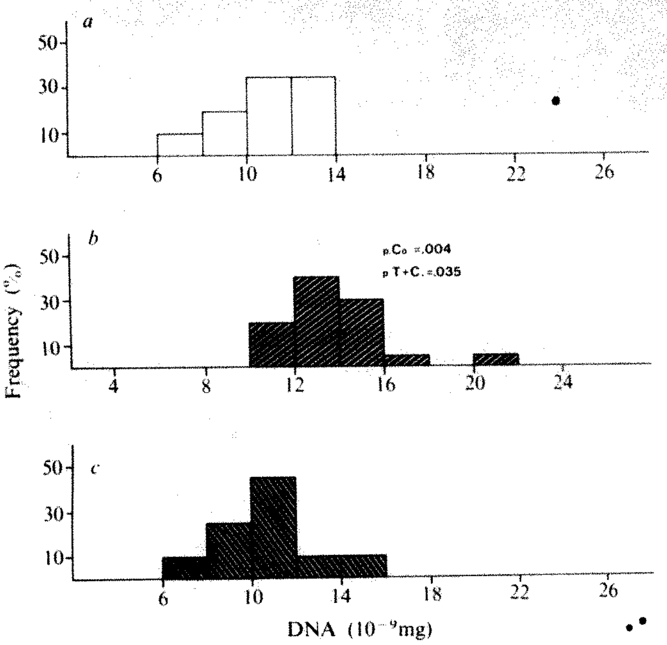

Fig. 4 DNA content (Feulgen microfluorometry) in metaphases of fibroblastic cells ($n=60$) from, *a*, 140 d control hamster lung culture; *b*, 132 d after six exposures to three puffs each of fresh cigarette smoke without a charcoal filter, and, *c*, to four puffs each with a charcoal filter.

illustrated in Figs 4 and 5. Although after exposure to smoke from cigarettes without charcoal the DNA content in metaphase and telophase was significantly greater and showed more variability than that of controls, there were no significant differences in the DNA content of cultures exposed to smoke from cigarettes with charcoal.

It seems, therefore, that the exposure of hamster lung cultures to a cigarette smoke with reduced gas vapour phase constituents and low SH reactivity not only produces less damage to the cells, but also less abnormal growth and disturbance of DNA complement in the chromosomes than smoke with greater amounts of gas vapour phase constituents and a higher SH reactivity. The observation, that these differences were found although both types of smoke had essentially the same amount of particulate matter (Table 1), indicates that gas vapour phase constituents contributed to the abnormalities. It has been reported that cigarette smoke inhibits SH-dependent enzyme systems[14–16], and that free radicals are responsible for this phenomenon[17]. The finding that free radicals are present in the gas vapour phase of cigarette smoke[18], not only supports the concept of the importance of gas vapour phase constituents for abnormal growth[1–6], but it also indicates that a disturbance of SH equilibrium in cells and tissues may be one of the steps in the sequential changes. On the other hand the observation that, even after the reduction of gas vapour phase components and of SH reactivity, the hamster lung cultures still showed some alterations, indicates the contribution of particulate matter of cigarette smoke to the occurrence of cellular abnormalities. In other words, the reduction of either gas vapour phase alone or of particulate matter alone does not yield a smoke which is harmless to cells in lung cultures.

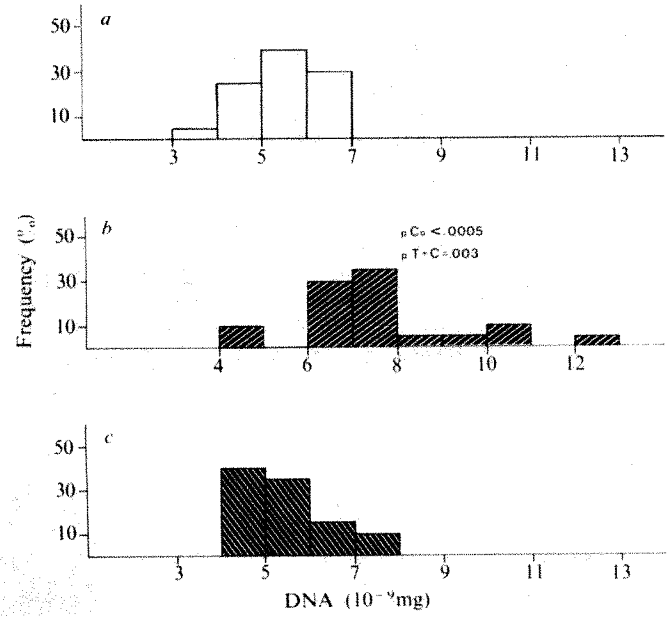

Fig. 5 DNA content (Feulgen microfluorometry) in telophases of fibroblastic cells ($n=60$) from, *a*, 168 d control hamster lung culture; *b*, 120 d after fourteen exposures to three puffs each of fresh ciagarette smoke without a charcoal filter and, *c*, to four puffs each with a charcoal filter.

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Poikilothermia and Susceptibility of Suckling Mice to Coxsackie B1 Virus

INFECTIONS with most types of Coxsackie virus are fatal to newborn mice in normal conditions. This susceptibility decreases sharply with age and, when the mice are a few days old, the infection runs a subclinical course[1]. As yet no satisfactory explanation of this phenomenon has been offered.

A greater susceptibility to some virus infections occurs if the temperature of the host is reduced and adult mice which are made hypothermic become susceptible to Coxsackie virus[2].

In most newborn animals there is a physiological hypothermia and poikilothermia[3]. We therefore studied the possible influence of body temperature on the susceptibility of newborn mice to Coxsackie virus.

The body temperature was measured in suckling mice varying in age from 12 h to 10 d. The mice were kept in cages held at room temperature (22 to 24°C). The body temperature was measured in the peritoneal cavity by insertion of a thermister. Each point in Fig. 1 indicates the average temperature of thirty to fifty suckling mice. The temperature was measured of mice taken directly from the nest with the mother on the nest, and it was ensured that she had stayed there for a period of at least 10 min. The temperatures measured 10 min and 20 min after the mother had been taken away from the nest are also shown. The babies were left piled just as when the mother normally leaves the nest. Each mouse was used for measuring one temperature only.

As the influence of poikilothermia on the course of infection is obviously related to the behaviour of the mothers, this was followed for a total of 6,360 min; twenty-two litters for 180 min in daylight and twenty litters for 120 min in twilight. The mothers were found to be away from the nest for an average total of 49% of the time (55% in twilight and 45% in daylight). Absences for periods longer than 10, 20 and 30 min, respectively, were recorded as 42%, 31% and 20% of the time. Concerning these longer absences, no significant differences could be found between daylight and twilight.

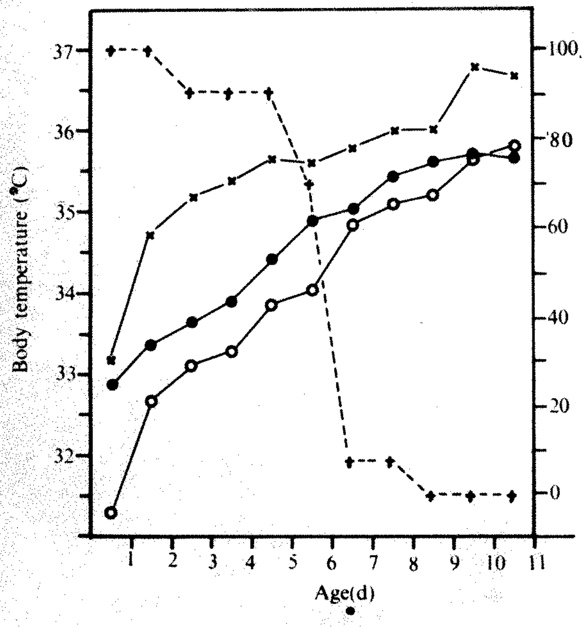

Fig. 1 Body temperature in suckling mice of varying age. The temperature was measured on mice taken directly from the nest with the mother on the nest, and it was ensured that she had stayed there for at least 10 min (×). The temperatures measured 10 min (●) and 20 min (○) after the mother had been taken away from the nest. The mortality in relation to the age at which the mice were infected (+).

The hypothermia and, particularly, the poikilothermia found in newborn mice thus decreased within a few days. At an age of 9 to 10 d, the mice had a temperature, with their mother on the nest, just as high as adult mice. The poikilothermia decreased especially after about 6 d. The effect of this decrease was pronounced, particularly when the mothers were kept away for relatively long periods, and is in agreement with previous results[4].

The mortality of suckling mice caused by Coxsackie B1 virus in relation to the age at which the mice were infected was also studied and is given in Fig. 1. The mice were infected intraperitoneally with a dose of 60 LD_{50} (titrated intracerebrally in 1-d-old mice). An abrupt decrease in mortality occurred around day 6, and after day 8 no mortality was seen with this dose of virus. This result is also in agreement with a previous report[5].

Table 1 Results of various experiments in which infected and uninfected newborn mice were placed at ambient temperatures of 22 to 24° C and 34° C.

Experiment No.	Inoculation: Age (d)	Inoculation: Virus dose	Inoculation: Route	Mortality at ambient temperature: 22 to 24° C	Mortality at ambient temperature: 34° C
1	1.5	$60\times LD_{50}$	Intracerebral	6/7	1/7
2	0.5	$60\times LD_{50}$	Intracerebral	6/7	3/7
3	1.5	$60\times LD_{50}$	Subcutaneous	7/7	1/7
4	2	$60\times LD_{50}$	Intraperitoneal	12/12	1/12
5	2	$60\times LD_{50}$	Intraperitoneal	10/10	3/20
6	1.5	$60\times LD_{50}$	Intraperitoneal	5/5	1/5
7	3	$60\times LD_{50}$	Intraperitoneal	10/10	3/25
8	3	No virus	—	—	0/20
9	1.5	No virus	Phosphate buffered saline (intraperitoneal)	—	4/20

To study the mortality of newborn mice in which the body temperature was stabilised around the lowest body temperature of the baby mice which survived, that is, 9 to 10-d-old babies, the cages with the mothers and their 1 to 3-d-old babies infected with a dose of 60 LD_{50} Coxsackie B1 virus were placed in an incubator at 34° C. The average body temperature of newborn mice kept in these conditions was found to be 35.8° C throughout the experiment. At this temperature, the suckling mice were lying free in the cage and were never found nesting. Table 1 shows the results of various experiments which revealed a pronounced reduction in mortality in mice with this body temperature throughout the course of infection.

Thus a correlation between poikilothermia and susceptibility to Coxsackie B1 virus was found. The higher temperature in the older mice might inhibit viral infection by decreasing virus replication in infected cells or by potentiating host defence mechanisms. Obviously the destruction of cells by growth of virus may also be different in hypothermic and normothermic conditions. Further evaluation of these possibilities is in progress in this laboratory.

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Interaction of Mercurials with Salmon Serum Lipoproteins

ALTHOUGH the toxic effects of mercury and its derivatives are well known[1–7] relatively little is known about the transport mechanisms involved in its distribution throughout the body. One possible means could be the serum lipoproteins through reactions involving lipids and proteins. Various metal ions bind with phospholipids[8]; for example, calcium ions bind to anionic sites of phospholipids in lipid monolayers[9]. In addition, phospholipids provide negatively charged sites for calcium ion exchanges in biomembranes[10] and contribute to the transport of ferrous ions in biphasic systems[11]. These findings, together with the fact that mercurials bind with negative sites of proteins, led us to explore the possibility that mercurials are actively sequestered by the serum lipoproteins.

We have now demonstrated that inorganic mercury ($^{203}HgCl_2$) and organic mercury ($CH_3{}^{203}HgCl$) compounds readily bind with the major classes of serum lipoproteins of sockeye salmon (*Oncorhynchus nerka*). The role played by lipid in these interactions is discussed here in reference to data obtained with a $CHCl_3 : H_2O$ model system.

Blood was drawn with a 3 ml syringe from the caudal vein of juvenile salmon 12–15 h after feeding. The serum was obtained by centrifuging the blood for 5 min at 1,500*g* to sediment the red cells. EDTA was added to the serum to give a concentration of 5 mg per 100 ml. The lipoprotein density classes were obtained by ultracentrifugation[12]. The lipoproteins (VLDL, LDL and HDL) and their apoproteins[13] were dialysed exhaustively against 0.01 Tris buffer ($pH = 7.2$) containing 0.15 M NaCl and 0.005 M NaN_3. VLDL is very low density lipoprotein of $d < 1.006$ ml^{-1}; LDL is low density lipoprotein of $d = 1.006 - 1{,}063$; HDL is high density lipoprotein of $d = 1.063$ to 1.21 g ml^{-1}. Apo VLDL, apo LDL and apo HDL are delipidated apoproteins of VLDL, LDL nad HDL. The lipoproteins and the apoproteins were diluted to volume so that the final concentrations approximated those present in the serum (Table 1). Protein was determined by the method of Lowry[14] and thin-layer chromatography was used to separate lipids[15,16] isolated from the lipoproteins. The protein : lipid ratios of the lipoproteins were as follows (w/w): VLDL (9 : 91), LDL (17 : 83) and HDL (46 : 54). The phospholipids in the VLDL, LDL and HDL represented 17, 36, and 55% of the total lipids, respectively.

The incorporation of the mercurials into the lipoproteins and apoproteins was examined at 10° C by placing 5.0 ml of lipoprotein or apoprotein solution (Table 1) inside a dialysis bag (4,000–6,000 molecular weight unit pore size) containing 0.1 ml of 15.07 μM $^{203}Hg^{2+}$ or $CH_3{}^{203}Hg^+$. The final concentration of the mercurials was 3.07×10^{-1} μM 0.06 p.p.m. The levels of Hg in *O. nerka* were shown to vary from 0.01–0.1 p.p.m. (private communication with E. J. Gauglitz jun.). The bag was then placed in 50 ml of buffer solution containing the same concentration of mercurial. The top of the bag was pressed shut with a rubber stopper against the neck of the flask and the solution was stirred continuously. Aliquots (1 ml) were taken from inside the bag and from the surrounding medium. Each aliquot was assayed for radioactivity and the differences between the d.p.m. ml^{-1} inside and outside represented the ^{203}Hg associated with the lipoproteins and apoproteins. After maximum incorporations, the solutions were transferred into fresh bags which were placed into 125 ml suction flasks containing Hg-free buffer. Fresh buffer flowed into the system at a rate of 1.0 ml min^{-1} and the excess was allowed to flow out of the side arm. The release of ^{203}Hg by the lipoproteins and apoproteins was determined as described for the incorporation studies. No evidence was found for amino acid residues[14] or phospholipids[15–17] outside the membrane that could possibly bind with the mercurials released from the lipoproteins.

The role of lipids in sequestering the mercurials was examined with a $CHCl_3 : H_2O$ system. The incorporation of $^{203}Hg^{2+}$ and $CH_3{}^{203}Hg^+$ was followed by placing 1.5 ml of fresh glass distilled $CHCl_3$ containing 1.25 μmol of lipid in a glass centrifuge tube equipped with a 'Teflon'-lined screw cap. Then 1.0 ml of buffer solution containing 3.07×10^{-4} μmol of radioactive metal ion was added. The solution was shaken vigorously for 30 s and then shaken in a water bath (14° C) for 2.0 h. The tubes were centrifuged for 15 min at 4,000 r.p.m. in a clinical centrifuge. Aliquots were taken from both phases and assayed for ^{203}Hg. The $CHCl_3$ phase was then extracted with 1.0 ml aliquots of buffer solution to determine whether mercuric or methylmercuric ions were released into the aqueous phase. The presence of radioactive ions in the aqeuous phase would indicate whether the metal-lipid complexes were readily dissociable. Duplicate runs were conducted in each experiment and the results were comparable within an error of ±3%.

The partitionings between the $CHCl_3$ and the buffer solutions were repeated with solutions containing Hg^{2+} and ^{14}C-labelled phospholipids to determine whether the metal-phospholipid complexes entered the aqueous phase. It was shown that no more than 2% of the ^{14}C was associated with the aqueous phase in each extraction. Thus, the Hg-phospholipid complexes were not removed from the $CHCl_3$ to a significant extent.

Lipids of high purity (>99%) from commercial sources were analysed by thin-layer chromatography[15,16]. No impurities were found. Phospholipids—phosphatidyl choline (PC), phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS)—were from bovine sources. The mercurials were obtained from International Chemical and Nuclear Corp., Irvine, California.

Evidence is presented to show that mercuric and methylmercuric ions are sequestered by the VLDL, LDL and HDL of fish serum under physiological conditions (Table 1). The fact

Table 1 Incorporation of Mercurials into Serum Lipoproteins of Salmon (*O. nerka*)

Mercurial	Lipoprotein density class	Mg lipoprotein ml^{-1}	Mg protein ml^{-1}	% Incorporation (maximum)	% Released*
$^{202}HgCl_2$	VLDL	1.67	0.16	22	42
	apo VLDL		0.17	2.7	21
	LDL	2.46	0.40	27	50
	apo LDL		0.40	5.9	39
	HDL	2.38	1.04	22	23
	apo HDL		1.06	25	2.8
$CH_3{}^{203}HgCl$	VLDL	1.67	0.16	11	28
	apo VLDL		0.17	3.3	39
	LDL	2.46	0.40	13	46
	apo LDL		0.40	5.2	27
	HDL	2.38	1.04	35	38
	apo HDL		1.06	35	38

The procedure is described in text.
* Percentage released in a 24 h period from the maximum incorporation obtained.

Table 2 Interaction of $^{203}HgCl_2$ and $CH_3{}^{203}HgCl$ with Lipids: Metal-Lipid Complexes Formed in $CHCl_3$ Solution

Lipids	Lipid in $CHCl_3$ (μmol)	Mercurial in aqueous phase (μmol)	Metal lipid complexes formed in $CHCl_3$ phase (μmol $^{203}Hg^{2+}$)	(μmol $CH_3{}^{203}Hg^+$)
Phosphatidyl choline	1.25	3.07×10^{-4}	1.4×10^{-4}	$< 0.02 \times 10^{-4}$
Phosphatidyl ethanolamine	1.25	3.07×10^{-4}	1.6×10^{-4}	$< 0.02 \times 10^{-4}$
Phosphatidyl serine	1.25	3.07×10^{-4}	1.7×10^{-4}	$< 0.02 \times 10^{-4}$
Cholesterol	1.25	3.07×10^{-4}	$< 0.02 \times 10^{-4}$*	$< 0.02 \times 10^{-4}$
Oleic acid	1.25	3.07×10^{-4}	$< 0.02 \times 10^{-4}$	$< 0.02 \times 10^{-4}$
Triolein	1.25	3.07×10^{-4}	$< 0.02 \times 10^{-4}$	$< 0.02 \times 10^{-4}$

The procedure is described in the text. * Limit of sensitivity: 0.02×10^{-4} μmol.

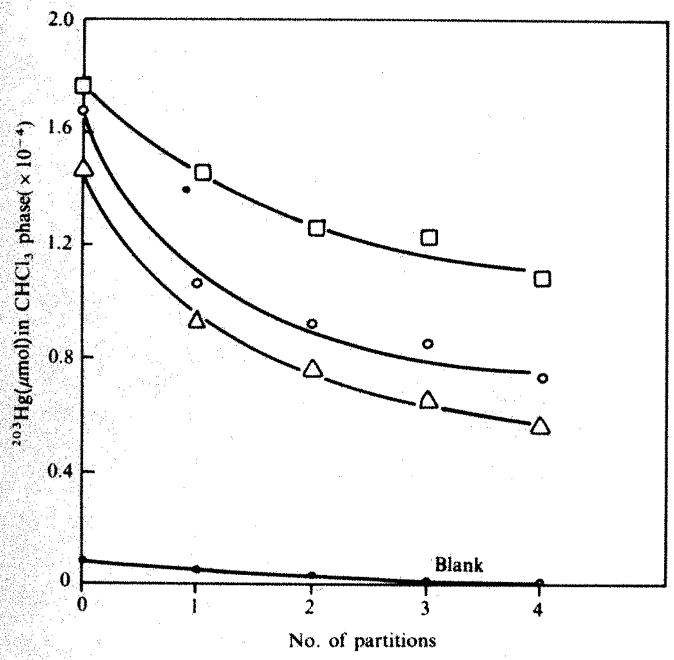

Fig. 1 The release of mercuric ions by phospholipid after partitioning with buffer. Procedure is given in text. □, Phosphatidyl serine; ○, phosphatidyl ethanolamine; △, phosphatidyl choline.

that considerable amounts of the bound $^{203}Hg^{2+}$ and $CH_3{}^{203}Hg^+$ were freely dissociated from the metallolipoproteins on removal of unbound mercurials suggests that the reactions between the mercurials and sites on the proteins may have been partly responsible for the weak bonds formed between the mercurials and the lipoproteins. Nevertheless, the major differences in the uptake and release of the ^{203}Hg between the lipoproteins and the apoproteins (Table 1) imply that lipids also played an important role in the interactions.

The studies with the $CHCl_3 : H_2O$ model system showed that PC, PE and PS readily bind and release Hg^{2+} (Table 2, Fig. 1). Thus, phospholipids form readily dissociable complexes with Hg^{2+} in $CHCl_3$ solutions. The active incorporation of $^{203}Hg^{2+}$ by PC, PE and PS suggests that phospholipids may play an important role in binding this ion in the lipoproteins. Moreover, phospholipids may well release the bound Hg as the Hg^{2+} concentration of the surrounding medium is decreased.

No evidence was found to indicate that neutral lipids or fatty acids participate directly in the binding of Hg^{2+}; however, the involvement of these lipids in sequestering Hg^{2+} in the lipoproteins cannot be ruled out.

The data did not indicate that CH_3HgCl binds with either the phospholipids, neutral lipids or fatty acids examined (Table 2). Thus, the interactions of methylmercury with the lipid portions of the lipoproteins (Table 1) may be related primarily to the high lipid solubility of this molecule[18]. In fact, considering the amounts of methylmercury incorporated into the lipoproteins and apoproteins, it seems that the high lipid content of the VLDL and LDL (Table 1) may account for the greater incorporation of $CH_3{}^{203}Hg$ into these lipoproteins relative to their apoproteins in comparison to HDL and apo HDL. It was suggested previously that the greater toxicity of methylmercury in comparison to inorganic mercury is due to the greater lipid solubility of the alkylated derivative[1].

The present initial studies implicating the serum lipoproteins in sequestering mercurials raises important questions about the significance of such interactions. Do mercurials disrupt the surface charge distributions or alter other structural features of the lipoproteins so that their physiological or immunological properties[19] are impaired. Detailed studies directed toward the possible role of the serum lipoproteins in the transport of the mercurials seems appropriate. Further, it would be interesting to know whether the observed interactions of the serum lipoproteins with mercuric and methylmercuric ions are intimately related to the toxic effects of these mercurials on vital organs, such as the brain.

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Is the Slow Worm a Batesian Mimic?

THE phenomenon of Batesian mimicry, where an edible animal gains an advantage by mimicking a distasteful or dangerous animal showing a warning pattern, is well known and has been described by many authors[1–4]. The advantage gained by a mimic decreases if the mimetic form becomes too common, since a predator may learn that the warning signal mimicked is a sign of edibility rather than danger; thus selection for the mimic is density-dependent which, as shown by Haldane and Jayakar[5], will often lead to a balanced polymorphism where both mimetic and non-mimetic forms are retained in the population. Matessi and Cori[6] suggest that mimetic inheritance is often sex-limited so that the expression of mimicry is limited to one sex (usually the female according to Sheppard[3]) although individuals of the other sex may have the mimetic genotype. It is suggested here that the slow worm (*Anguis fragilis* L.) has a form of colouration exhibited by the young and some adult females which is a mimic of the warning pattern of the adder (*Vipera berus* L.).

Although adders are very variable in their ground colour and markings, one characteristic that immediately distinguishes them from the other British snakes is the dark, zigzag line down the centre of the back. The pattern may be broken up or more like a continuous stripe, but it is still the most distinctive feature of the snake and almost certainly acts as a warning to potential enemies (although the pattern may act as camouflage in dead bracken (E. P. Killips, personal communication)). Certainly, both Smith[7] and Prestt[8] say that the chief enemy of the adder is man and, although adders are sometimes taken by the buzzard (*Buteo buteo* L.) and the hedgehog (*Erinaceus europaeus* L.), they seem to have few natural enemies. Prestt[8] found that even 3-d-old adders could inflict a poisonous bite but that generally when domestic animals were bitten they were not killed, suggesting that animals could learn, by experience, to avoid adders without the lessons proving fatal. Reid[9] confirms that, as well as a prey-killing bite, venomous snakes generally have a defence or warning bite which injects little or no venom but allows the snake to escape.

Slow worms do not usually grow as large as adders and have upper parts which vary considerably in ground colour, a variation probably associated with their habitat. All newborn slow worms have a thin black zigzag or stripe down the middle of the back but, as the animals reach sexual maturity, all males and some females lose the stripe. It seems likely that the females which retain the stripe are mimicking the adder, and the presence of the dimorphism in the females but not the males supports this hypothesis. The question that follows is, when will the mimicry be of any advantage?

The slow worm is not often seen in daylight, preferring to hide under stones, logs or leaves or even burrowing in light soil. It does appear in the day, however, in early spring when mating, and in the late summer when pregnant females bask in the sun. Both the adder and the slow worm are ovoviviparous, an uncommon feature among the Squamata, so the eggs are less likely to fall victim to predators and the mother can warm the eggs inside her by basking, or shelter them from extremes of heat and cold by taking cover. The mother and young are probably most vulnerable near the time of birth, so if the female adder gains some protection from its aposematic colouration at this time, the female slow worm and her young might also be protected by mimicking the warning. The loss of the distinctive pattern by all the males and some of the female slow worms shows the dynamic balance that must exist between selective advantage and frequency of the mimetic form.

If the maximum advantage is to be gained from the mimicry, the active periods of mating and birth in the slow worm should correspond with those of the adder. Figure 1 shows the activities through the year of the adder, the slow worm and viviparous lizard (*Lacerta vivipara* Jacquin) adapted from Smith[7]. Mating in the slow worm tends to occur towards the end of the adder's mating period but birth takes place over a very short period of time corresponding to the peak birth period of the adder. By way of comparison, the viviparous lizard gives birth to its young very much earlier, suggesting that the slow worm has evolved such that the mimicry gives maximum advantage at the most important time.

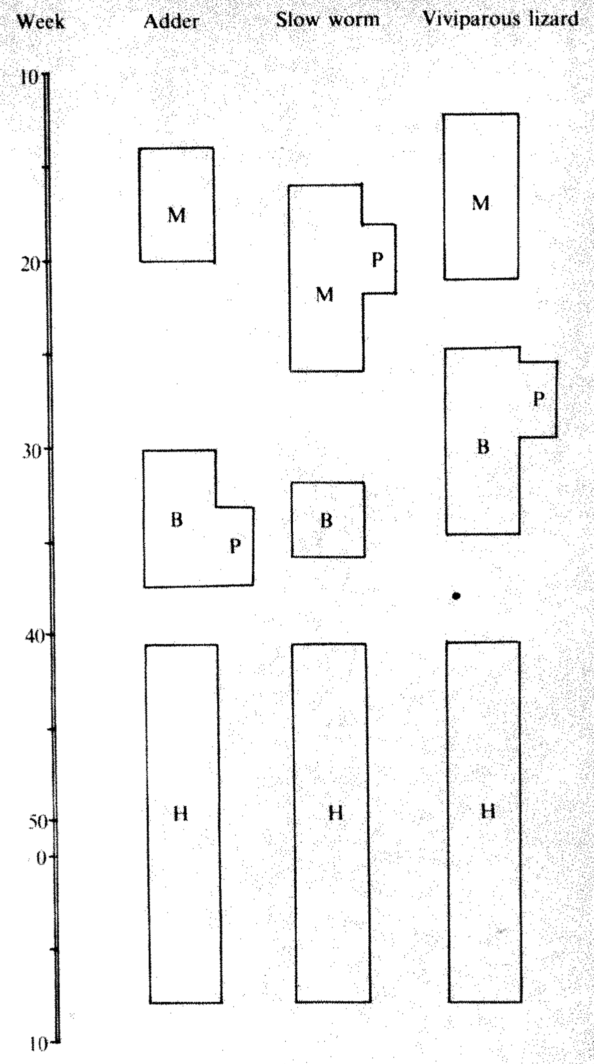

Fig. 1 Activities throughout the year of the adder, slow worm and viviparous lizard. *H*, Hibernation; *M*, mating; *B*, birth; *P*, peak period.

The hypothesis that the slow worm is a Batesian mimic of the adder is difficult to test experimentally, although a laboratory experiment using adders, slow worms and assorted predators could be devised. The evidence presented in Fig. 1, however, supports the hypothesis, and it is hoped to estimate the selective value of the mimetic form by mark recapture as described by Manly[10]. Data are currently being collected to discover whether the frequency of the patterned form is related to the frequency of adders, and of particular interest are areas where slow worms but not adders are found, such as Radnor, South Lincolnshire, the Outer Hebrides and the Channel Islands. Islands should be particularly informative since the effects of natural selection in the absence of migration can be seen there. N. Jee and R. Brehaut report that they have never observed an adult slow worm with a dark stripe on Guernsey, but they have not been looking out for the pattern so it could have escaped their notice; however, their evidence supports the hypothesis suggested here.

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Differential Climatic Selection in Natural Population of Land Snail *Cepaea nemoralis*

DURING a study of the energetics of a population of the polymorphic land snail *Cepaea nemoralis* L. on the sand dunes at Braunton Burrows, North Devon, numbers of dead and dying snails were collected during the daylight hours in the summer. They showed similar symptoms, being unable to retract the foot which had become dried, discoloured and unresponsive to touch stimuli. Some animals were unable to retract the optic tentacles and in some, the genitalia protruded. Snails found in this condition, although alive (as evidenced by sensitivity of the mantle area) early in the day, were usually dead by evening.

At first a parasitoid fly was suspected as the cause of death, but on incubating the dead animals, only two flies emerged: a specimen of the calliphorid *Sarcophaga nigriventris* and a muscid (*Fannia* sp.). Both are opportunist scavengers[1], but *Sarcophaga* has been found parasitising snails. Later experiments on the upper lethal temperature of snails produced symptoms identical to those found in the field and it was assumed that the animals had died as a result of high temperatures.

The animals were collected on two occasions in 1970 and six in 1971 from an area of known size (1,600 m^2). The total number collected and the number of deaths per week are shown in Table 1.

Table 1 Heat deaths in an area of 1,600 m^2

	Total dead snails collected	Deaths (week^{-1})
1970 1 Oct.	206	—
15 Oct.	30	15.0
29 Oct.	9	4.5
Total	245	Average 9.75
1971 29 April	25	5.0
13 May	16	8.0
24 June	15	2.5
22 July	10	2.5
29 July	4	4.0
16 Aug.	6	1.5
Total	76	Average 3.92

The mean population of adult snails in the area was 1,900 and the mean weekly survival rate in a sub-area used for a capture-mark-recapture study was about 0.9987, which compares with 0.9566 calculated by the Jolly[2] capture-mark-recapture census over the same area.

There is no clear seasonal trend in the mortality, but it has been shown[3] that almost any sunny day from March to September can produce temperatures at the snails' upper lethal limit near the grass surface. For short periods of exposure (0.5 h) the upper lethal temperature is about 43° C.

The frequencies of colour and banding morphs of the dead animals were compared with those prevailing in the population, based on live animals collected during random quadrat sampling. In this instance, a collection made from another area of the sand dunes (Site 2), with rather different morph frequencies is included. Table 2 shows the morph frequencies of the dead snails with the expected frequencies in brackets.

Table 2 Morph analysis of dead shells collected during 1970–1971

1970 Site 1	Yellow	Pink	Brown	Total
00000	0 (0.5)	3 (10.5)	12 (11.3)	15 (22.3)
00300	12 (12.7)	78 (81.1)		90 (93.8)
12345	10 (17.6)	130 (111.2)		140 (128.8)
Total	22 (30.8)	211 (202.8)	12 (11.3)	245
1971 Site 1	Yellow	Pink	Brown	Total
00000	0 (0.2)	0 (3.3)	1 (3.5)	1 (7.0)
00300	4 (4.0)	23 (25.2)		27 (29.2)
12345	4 (5.5)	44 (34.5)		48 (40.0)
Total	8 (9.7)	67 (63.0)	1 (3.5)	76
1970 Site 2	Yellow	Pink	Brown	Total
00000	5 (15.2)	27 (45.6)	1 (0.0)	33 (60.8)
00300	10 (21.3)	291 (258.1)		301 (279.4)
12345	4 (21.3)	169 (145.5)		173 (166.8)
Total	19 (57.8)	487 (449.2)	1 (0.0)	507

The differences between the observed and expected frequencies may be tested by a log likelihood ratio test[4]; the G statistic degrees of freedom and probabilities are shown in Table 3.

Table 3 Comparison of observed dead shell morph frequencies

	G	d.f.	P
1970 Site 1	15.48	5	0.01–0.005
1971 Site 1	6.76	3	0.1–0.05
1970 Site 2	52.49	5	<0.005

In all cases, differences between the observed and expected frequencies are apparent and are highly significant in the cases of Sites 1 and 2 in 1970. These two collections were much larger than the 1971 one and included shells which had been dead for some time.

The trend in all three collections is similar: lower numbers of unbanded and yellow shells than expected and correspondingly higher numbers of banded and pink shells, suggesting that yellow and unbanded animals are more resistant to high temperature stress than pink and banded animals. Schnetter[5] suggested that physiological selection favoured unbanded yellow shells of *C. hortensis* in dry sunny situations, and in laboratory experiments, Boettger[6] (in *C. hortensis*) and Lamotte[7] found yellow and unbanded animals to be more resistant to high temperatures. Yellow-shelled animals selected a higher temperature in a temperature gradient than pink-shelled ones in a study by Sedlmair[8].

It is also interesting to note that laboratory experiments on the albedo of empty shells[3] showed that yellow unbanded shells reached a temperature of 1 to 2° C lower than that attained by pink banded shells (especially where the bands were fused producing an effectively black shell) under the same radiation conditions. This small difference may be important in producing differential survival when temperatures are near the upper lethal temperature.

The polymorphism of sand-dune populations of *C. nemoralis* has been discussed by Cain[9]. He suggests that the overall distribution of morph frequencies is consistent with the action of visual selection by predators. On the present site, thrush predation occurred only in the winter months on buried, hibernating snails, and thus with no opportunity for visual selection. Climatic factors are indicated by Cain to be of some importance, notably the relationship between high frequencies of the dark brown morph and areas where cold air accumulates at night, but also the higher frequency of snails with yellow mid-banded shells on sunny south-west facing slopes as opposed to higher frequencies of snails with pink five-banded shells on north and east facing slopes. My findings provide what seems to be the first direct evidence[10] of differential climatic selection in the field and support Cain's concclusion that climatic selection may over-ride visual selection in the maritime duneland situation.

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Habitat selection in titmice

HABITAT selection has important ecological and evolutionary consequences for birds as for other animals[1–4], yet although there is abundant evidence from natural observation that it occurs, little is known of the cues by which habitats are recognised, nor the role of experience in determining such preferences[5–10].

In Europe the coal tit (*Parus ater*) is primarily conifer-dwelling whereas the closely related blue tit (*Parus caeruleus*) lives mostly in deciduous woods[11]. There is some overlap, and where this occurs there are differences in habitat utilisation[12–14]. The experiments described here were designed to show if hand-reared birds with no experience of vegetation would show any preference for the type of vegetation in which they are usually found. This type of experiment has been conducted with rodents[10], and with birds[15], but with the latter the results were not clear, so that the question of whether 'naive' birds can make this distinction remains.

The experiments were carried out with a mixed group of naive birds, eight blue tits and six coal tits. They were taken from the nest 10 d after hatching and raised together until the tests. At 8 weeks the birds were presented with a composite branch hung from the ceiling of the aviary. The branch was made from two smaller ones lashed together side-by-side and each about 7 feet long. One was of Oak (*Quercus robur*) and the other of Scots Pine (*Pinus sylvestris*). The birds showed immediate interest in the branches and searched them assiduously for about an hour after their introduction; the branches on which the birds were perched were recorded every minute. The experiment was conducted twice, a few days apart. The results (experiment *a*, Table 1 and Fig. 1) show that there is a significant difference in preference, each species preferring that type of tree in which it is most often found in the wild.

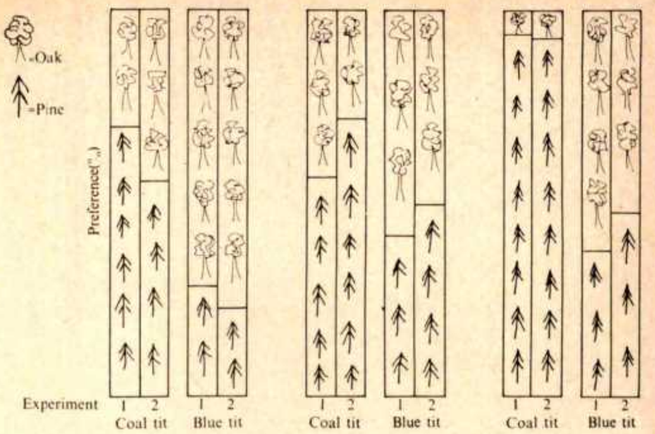

FIG. 1 Percentage preference for oak and pine by the coal tit and blue tit in the three pairs of experiments. *a*, with complete branches; *b*, branches stripped of leaves and, *c*, leaves alone.

This method has several advantages: because the twigs of the two branches are closely interwoven, one can be sure that differences in preference cannot reflect differences in the favoured part of room, for instance with reference to shade or temperature. Nor can it have been produced by any tendency to flock with or avoid members of their own or the different species, as a bird attracted to flock with or avoid another would have had both species of branch within a few inches. Imitation[16] is unlikely to have occurred because the birds were frequently out of sight of one another on the branch, and when feeding they seemed to observe the other birds very little. As identical branches were presented for both species of birds, differences in the amount of twig available for perching, or food available on the branches could not have produced the results.

In previous years experiments conducted with individual birds gave variable results because the branches die quickly, and therefore different pairs of branches had to be used for

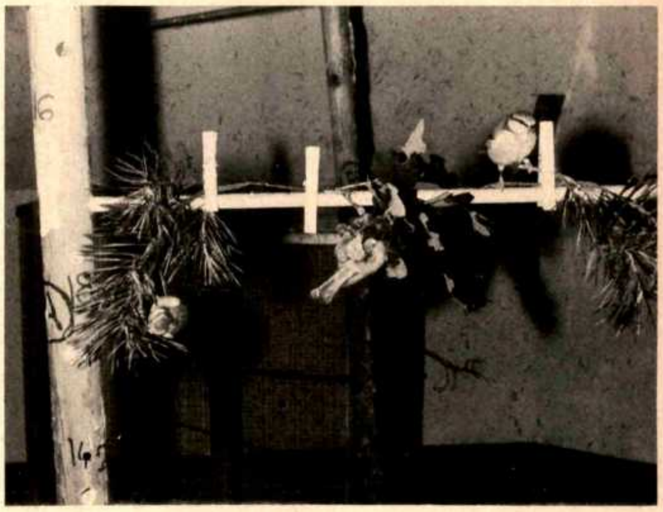

FIG. 2 Arrangement of leaves alone in experiment *c*.

TABLE 1 Table of bird-records for coal tits and blue tits perched in oak and scots pine in the three pairs of experiments.

		Oak	Pine	% Pine	Oak	Pine	% Pine	Oak	Pine	% Pine
Experiment 1	Coal tit	27	51	65	63	85	57	1	16	94
	Blue tit	106	44	29	116	83	42	42	26	38
			p*			p†			p*	
Experiment 2	Coal tit	67	84	56	21	54	72	2	27	93
	Blue tit	159	48	23	61	62	50	44	40	48
			p*			p†			p*	
		Complete branches (a)			Branches stripped of leaves (b).			Leaves alone (c)		

* Significance level <0.1% on 2 × 2 χ^2 test.
† Significance level <1% on 2 × 2 χ^2 test.

each bird. It was noted that all individuals of each species seemed to show the same preference although it is possible that there were quantitative differences. The intense activity of the birds while searching meant that when successive observations were made on the same individual it was likely to be in a different region of the branch. There was no trend of increasing or decreasing preference with time for either species.

To examine the effect of leaves on the preference, two more experiments (*b* and *c*) were undertaken. In one the birds were presented with a composite branch stripped of leaves, in the other with leaves in a manner shown in Fig. 2. The leaves were pegged in bunches onto wooden perches. The results (Table 1) show that there is a significant difference in preference both for the bare branches and for the leaves of the tree in which they are usually found. Figure 1 illustrates that in the last experiment the blue tits distributed their visits more evenly between the two branch types than the coal tit who landed very little in oak leaves.

These experiments show that these two bird species can actively select their usual habitat in the absence of any previous experience of it. To what extent this preference may be modified by subsequent experience remains to be seen.

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Coral Growth Related to Resuspension of Bottom Sediments

THE determination of coral skeletal growth, from both a biological and a geological standpoint, has long presented difficulties in ease and accuracy of measurement. As a consequence, factors which limit growth are only beginning to be investigated. Radiometric methods using natural radioactive series nuclides have recently been used to determine the growth rate of certain corals[1,2]. In addition, X radiographs coupled with ^{90}Sr-induced autoradiographs have demonstrated that at least some hermatypic corals record annual growth bands in their skeletons[3,4]. Once annual banding is confirmed, measurement of band widths allows precise determination of yearly growth increments. It is thus possible to compare inter- and intraspecific coral growth rates from different areas with pertinent environmental variables and to educe possible correlations. We describe here an investigation of the effects of resuspension of bottom sediments on the growth rate, as determined by a ^{228}Ra technique and X radiography of *Montastrea annularis* in different parts of Discovery Bay, Jamaica. Such a study is unique both because the annual nature of growth bands is established for the most important reef-forming coral in the Caribbean[5] and because radiometric and radiographic techniques are extended to measure growth rates as a function of environmental parameters.

The effect of sedimentation on coral growth has never been extensively analysed. Marshall and Orr[6] demonstrated that although relatively high rates of sediment fouling will kill coral species, most corals can withstand a low sediment input by active physical removal of the sediments. Yonge[7] states from findings in Professor T. Vaughan's notes that sand was removed, although slowly, from the surface of *M. annularis* with much mucus being extruded, and has discussed also a coral species' ability to clear itself of sand by distension of the coenosarc with water[8]. More recently, the self-cleaning ability of certain corals has been further discussed[9–11]. Constant energy expenditure by corals for removal of sediment particles might be expected to decrease growth rate. In addition, hermatypic corals are known to be dependent on light for skeletal growth[12], so that the decrease in available light intensity due to turbidity might also have a marked effect on growth rate.

After preliminary observations of the shallow rear zone and adjacent soft bottom fauna of the lagoon of Discovery Bay, we measured resuspension rates of bottom sediments in order to assess bottom stability and turbidity[13]. Three stations were established (Fig. 1) on the soft carbonate sediment bottom near the rear zone of the reef. Resuspension was measured in duplicate at each of the three stations by placing sediment traps 50 cm above the bottom and weighing the collected sediment. Weather conditions were identical throughout the experiment (3/16/73–3/24/73 for stations A and B, 3/17/73–3/25/73 for station C), and no significant variations in either salinity or temperature were observed between the stations

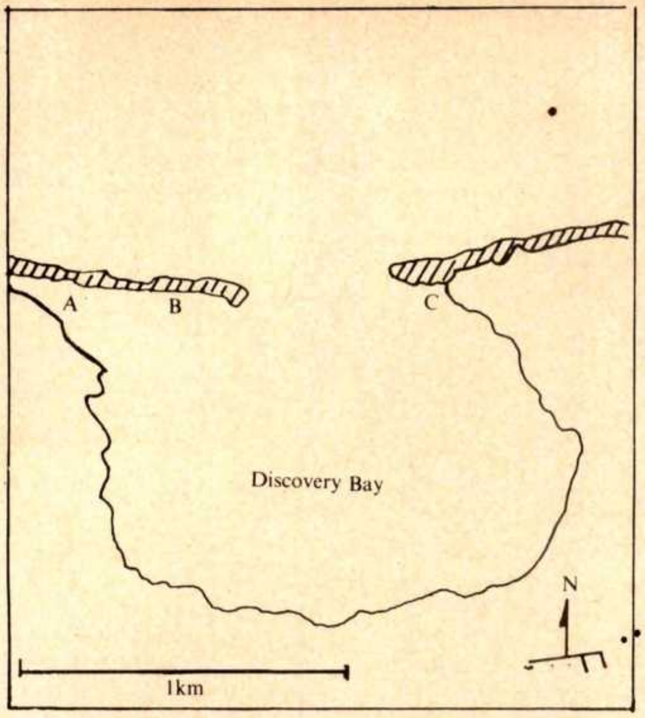

Fig. 1 Sketch map of Discovery Bay, Jamaica, showing station locations (A–C). Stippled area represents reef.

(R. C. A. and R. E. D., in preparation). The resuspension rates determined were thus an integrated measure of bottom instability under the following diurnal conditions: throughout the morning hours the lagoon is calm and clear, but by afternoon the Northwest Trades, which blow daily, have driven water turbidity to a maximum, and by evening the lagoon is again calm and clear and remains that way all night. Jackson[14] presents a number of environmental variables measured at the eastern part of Discovery Bay over the period of a year. His data indicate that March is characterised by turbidity values relatively lower than those for winter and slightly higher than those for summer. Our data suggest that a gradient of increasing resuspension from east to west exists in the Bay.

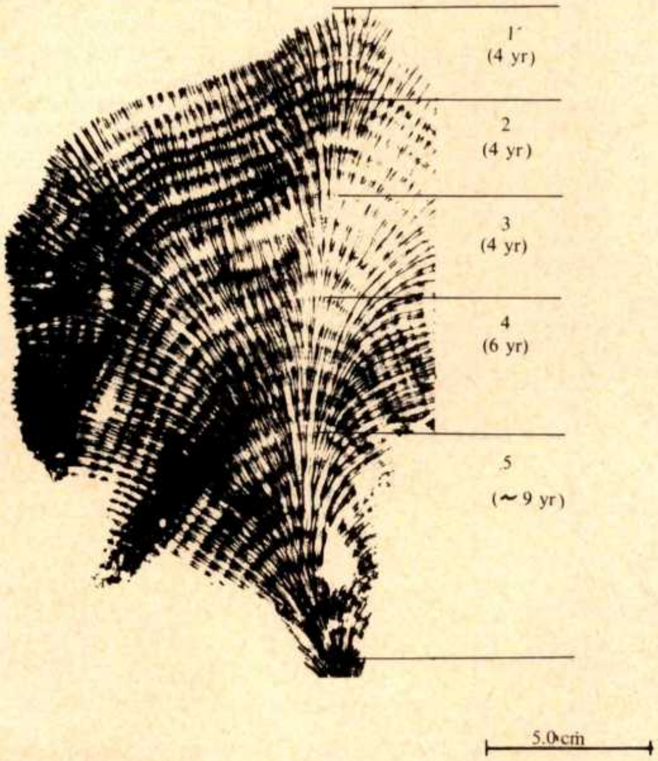

Fig. 2 X-radiograph positive of a specimen (A2) of *Montastrea annularis* from station A showing annual bands and locations of five samples for ^{228}Th analysis.

Coral heads of various species were collected from the rear zone of the lagoon at depths of 2–4 m, within 50 m of the station locations. *M. annularis* was chosen for intraspecific analysis because it is a representative species and also the major Caribbean reef framework coral[5] and has been shown previously to exhibit skeletal banding[3].

Specimens collected were hemispherical to semi-hemispherical, not larger than 50 cm in height, and taken only from areas of living reef. The corals were first sectioned into slabs about 2 cm thick on a rock saw. These slabs were oriented so as to include the point of highest relief on the actively growing coral surface and also the midpoint of the base. The slabs were prepared and X radiographed using the techniques of Knutson *et al.*[3] except that slab thicknesses of 0.3–0.5 cm were used. Band widths of each specimen were measured along several line transects drawn on the X-ray positive (Fig. 2), one transect always drawn so as to include the axis of maximum growth. Because of the imperfect hemispherical shape of specimens, the other transects were drawn for calculation of an 'average' estimate of upward growth. Table 1 gives the results of these measurements. Values for coral growth rate are weighted means derived from calculation of the means and standard deviations of each transect in a slab. The maximum growth rate given for each station is a weighted mean of transects along the axes of maximum growth of all corals at that station, and the 'average' growth rate of a station is a weighted mean of all transects. Weighted means and associated weighted standard deviations have been used to favour individuals that show a more even growth. This seems reasonable in a biological sense because we lack information on the history of each coral.

Table 1 Coral Growth Values for Stations and Individual Heads Collected at Each Station

Corals and stations	Number of transects	Average year/transect	Growth rate (cm yr^{-1}) weighted mean ± weighted σ
Station A			
A1	2	16	0.50 ± 0.059
A2	2	21	0.60 ± 0.082
A3	2	6	0.72 ± 0.055
Maximum (contains axis of maximum growth transects only)			0.65 ± 0.050
Average (contains all transects for station)			0.62 ± 0.036
Station B			
B2	3	6	0.82 ± 0.055
B4	3	6	0.83 ± 0.077
Maximum (contains axis of maximum growth transects only)			0.90 ± 0.063
Average (contains all transects for station)			0.82 ± 0.047
Station C			
C2	3	4	1.06 ± 0.085
C3	2	12	0.58 ± 0.108
C7	3	10	0.88 ± 0.075
Maximum contains axis of maximum growth transects only)			1.00 ± 0.082
Average (contains all transects for station)			0.88 ± 0.050

The determination of the rate of growth of *M. annularis* was done by α spectrometric analysis for *in situ* ^{228}Th growing in from ^{228}Ra which had been precipitated from seawater with the aragonitic coral skeleton. The chemical procedure was similar to that of Moore *et al.*[2]. Samples including sufficient growth bands to yield about 50 g were obtained for a specimen from station A by comparison of a slab with its associated X radiograph (Fig. 2).

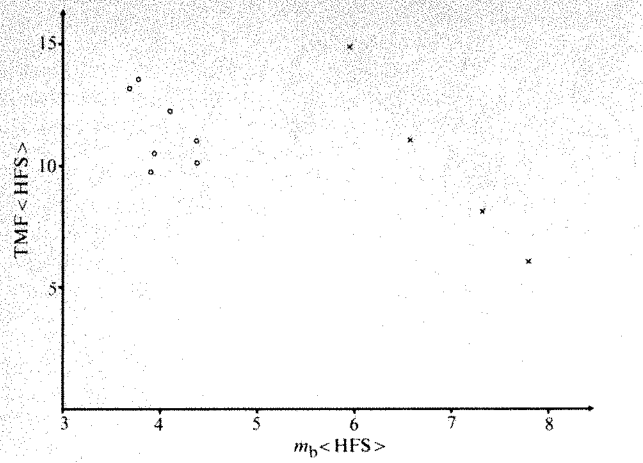

Fig. 3 ^{228}Th specific activities obtained for skeletal intervals shown in Fig. 2. The curve is the predicted ^{228}Th ingrowth for an initial ^{228}Ra value of 4.0 d.p.m. per 100 g coral.

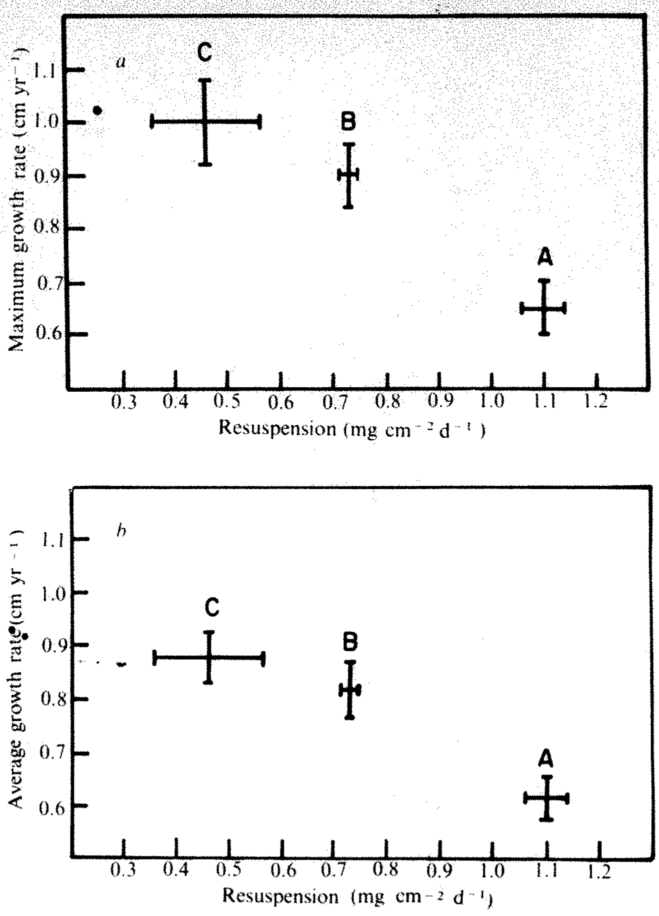

Fig. 4 *a*, Maximum and, *b*, average, upward coral growth (error bars$=2\sigma$) against ranges of resuspension values for each station.

The *in situ* ^{228}Th method depends on the assumption that the coral incorporates ^{228}Ra into its skeleton at a fixed ratio to Ca from the surrounding seawater, and that this initial ratio has been constant throughout the life of the coral. Analysis for ^{228}Th at time intervals defined by the growth bands should then conform to an ingrowth curve of ^{228}Th towards transient equilibrium with the initial ^{228}Ra specific activity. In Fig. 3 the 1 sigma counting uncertainties on the ^{228}Th analyses are entered at the midpoint of the time spans except for sample 5 which has been weighted towards younger values because of volume considerations in the triangular sample. The curve in Fig. 3 corresponds to the predicted ^{228}Th ingrowth for an initial ^{228}Ra specific activity of 4.0 d.p.m. per 100 g. Recent measurements of ^{228}Ra in Caribbean surface water[15] lie in the range 3.7 to 4.4 d.p.m. per 100 kg, so that *M. annularis* seems to exhibit little discrimination against Ra relative to Ca. However, a specimen of *Stephanocoenia* sp. taken at a depth of 25 m in Discovery Bay is reported[1] to have an initial value of 1.3 d.p.m. ^{228}Ra per 100 g. The reason for this difference is not known.

Resuspension values are plotted against both 'average' and maximum coral growth rates in Fig. 4. There is a definite inverse trend between resuspension values and coral growth in both cases with increased resuspension and lowered growth rates from east to west. The stress gradient thus defined results from both physical and biological factors. The North-east Trades couple with bottom stability factors such as benthic infaunal burrowing activities and sediment-binding algae (R. C. A. and R. E. D., in preparation). The standard deviation of both maximum and 'average' growth also decreases as resuspension increases, indicating that not only does high resuspension hinder coral growth, but also decreases the variability in growth. This suggests that the ability of the coral to respond to other favourable, or at least less limiting, environmental variables is reduced or overshadowed by high resuspension.

This study demonstrates the importance of sampling location in any study of coral growth rate. In selected areas in Florida and the Caribbean various workers (refs 3, 16–18, and I. G. MacIntyre and S. V. Smith, written communication from paper presented at 2nd Int. Symp. on Coral Reefs, Great Barrier Reef (1973)) have found a range of 0.50–1.7 cm yr^{-1} for skeletal growth of *M. annularis*. If all the rates are intercomparable, it is obvious that one species can have values ranging over a factor of three without gross morphological indications. Further, our data indicate that a factor limiting coral growth is closely associated with the intensity of resuspension of bottom sediments. Other factors, such as temperature, light availability, salinity and nutrient supply, are clearly also important. An extensive analysis on a reefwide scale would be very useful, not only in delineating average growth conditions but also in uncovering other possible limitations on coral growth. When these factors are understood, the effects of perturbation, whether by man or nature, can be assessed. Then problems such as the rate of recovery following growth disruption or retrieval of historical information based on 'dendrochronological' analysis can be approached.

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Possible Therapy for Capture Myopathy in Captured Wild Animals

CAPTURE myopathy (so-called overstraining disease) in wild animals has gained increasing prominence over the past decade as attempts to capture remaining nuclei of rare species for relocation and restocking, are rendered abortive by high death rates. The proportion of deaths is usually highest in the calves and in gravid females. High death rates in diminishing animal species such as tsessebe (*Damaliscus lunatus*) have occurred in spite of all precautions, such as the use of a helicopter to reduce the time between alerting the subject animal and the placement of a syringe containing suitable immobilising compounds.

Investigation on a number of species including blesbok (*Damaliscus dorcas*) and wildebeest (*Connochaetes taurinus*), has shown that an acute metabolic acidosis seems to be the causative factor in several at least of the manifestations of capture myopathy and death. A fall in the blood *p*H and attendant factors, namely base excess, buffer base, plasma and total bicarbonate, occur most precipitously in those animals pursued intensively for short distances of 1 to 2 km. The deaths, with symptoms of capture myopathy seem to be due mainly at least to acute and profound acidosis rather than, as was widely believed, to exhaustion and overstraining of muscles and vital organs.

The fall in blood *p*H continues after the animals have been captured. The crating of captive animals such as zebra (*Equus burchelli*) in itself causes acidosis, so that little or no abatement of the condition may be expected during subsequent transport.

We have treated the syndrome induced experimentally in nine wild zebra (weighing 200–250 kg) pursued over short distances. We infused them intravenously with 1,000 mEq of sodium bicarbonate dissolved in Normosol solution. Normosol solution (Abbott, Johannesburg) contains the following ions: Na^+, 140; K^+, 5; Mg^{2+}, 3; Cl^-, 98; acetate, 27; gluconate, 23 mEq l^{-1}. Approximate *p*H 7.4. This therapy was successful. Six animals were treated and all survived. The treatment, given over 5 to 10 min, was carried out 30 min after capture and resulted in immediate and spectacular amelioration of the predominant clinical symptoms such as dyspnoea and cardiac dysfunction: heart and respiratory rates were slowed, and the heart sounds became clearly audible and defined. Skeletal muscular spasm, as evident in untreated animals was prevented, and there was an immediate improvement in the animals' reactions and general condition.

The three untreated animals died within 12 h apparently of pulmonary oedema with *post mortem* findings exhibiting characteristic lesions of capture myopathy.

We do not wish to oversimplify the problem presented by capture myopathy in conservation measures, nor exclude genetic or acquired predisposition. It does appear, however, that at least aspects of the acute phase of the syndrome may be remedied.

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Ribonuclease activity of wheat leaves and rust infection

REMARKABLE changes in the levels and properties of ribonuclease (RNase) occur following the inoculation of a susceptible variety of flax (*Linum usitatissimum* L., var. Bison) with flax rust uredospores (*Melampsora lini* (Ehrenb.) Lev, race 3)[1]. The increase in RNase activity occurs in two distinct phases; an early phase between 2 to 4 d after inoculation (early RNase) and a later phase, beginning 5 d after inoculation (late RNase). These increases in RNase activity are accompanied by substantial changes in the properties of the enzyme including thermal stability, diethylpyrocarbonate-sensitivity and substrate preference[1]. Further studies (A. K. C., M. S., and L. A. S., unpublished) have revealed that these changes in the properties of RNase are due, at least in part, to the formation of new RNase molecules. These have kinetic and catalytic properties that are quite distinct from both of those 'host' RNase from healthy flax cotyledons and 'rust' RNase from flax rust uredospores or from rust mycelium grown axenically in a chemically defined medium. That changes in late RNase activity are specifically elicited by compatible host-parasite interactions is indicated by the appearance of late RNase which is not detectable in a resistant variety of flax (Bombay) inoculated with race 3 of *Melampsora lini*[1]. Furthermore, the characteristic bimodal increase in RNase activity and the accompanying changes in various properties of the enzyme have been found in a number of other rust-infected plants including pine and *Ribes* (A. E. Harvey, A. K. C., M. S. and L. A. S., unpublished), and mechanical injury of the healthy host tissues causes a significant increase in the level of RNase. The enzyme remains strictly 'host-type', however, no change in its physical or catalytic properties being detectable (ref. 1 and A. E. Harvey, A. K. C., M. S., and L. A. S., unpublished). Here we describe the effect of inoculation with uredospores of three races (56, 56A and Australian race 126 ANZ-6, 7) of wheat stem rust (*Puccinia graminis* (Pers.) f. sp. *tritici* Eriks. and E. Henn.) in causing quantitative and qualitative changes in the RNase activity of paired, near-isogenic lines of wheat (*Triticum aestivum* L.) derived from the variety Chinese Spring and carrying the alleles *Sr6* and *sr6* (refs 2 and 3), respectively. Both lines give susceptible reactions (infection type 4 (ref. 4)) with race 56A, independent of temperature. The homozygous recessive, *sr6*, imparts susceptibility (infection type 4) to races 56 and 126 ANZ-6, 7. The dominant allele, *Sr6*, confers a temperature sensitive reaction to these races; resistance is expressed (infection type 0 (ref. 4)) at 19 to 21° C but breaks down (infection type 4) at higher temperatures (25 to 26° C). A few intercellular hyphae are produced at each infection site at 19 to 21° C. These survive for several days and resume growth when infected plants are transferred to 25 to 26° C; but, in our experience, the longer infected plants are held at 19 to 21° C before transfer, the smaller are the number and size of the sporulating pustules to be developed.

The isolate of the Australian race, 126 ANZ-6, 7, was provided several years ago by Dr P. G. Williams and has been maintained by us. It was included in this study because it is readily grown in axenic culture[5,6] and is therefore a convenient source of mycelial RNase. The results indicate a positive correlation between increased late RNase activity and the ability of the host to support the growth of the pathogen, and for qualitative changes in the properties of both early and late RNase.

Wheat seedlings were grown in a growth chamber at 20° C with 16 h illumination at 800 to 1000 foot-candles and 8 h darkness daily. Fully-expanded primary leaves were inoculated by dusting with a mixture of uredospores and talc or talc alone (controls). After dusting, the plants were placed in a humidity chamber[1] for 12 h and then returned to the growth chamber. Batches of healthy and inoculated plants were transferred at intervals to a second growth chamber maintained at a daylight temperature of 26° C. The number of sporulating pustules (infection type 3–4) was recorded 1 or 3 d after they first appeared. RNase was extracted from excised primary leaves and partially purified by acidification at *p*H 5.0 as described before[1].

Figure 1 shows the RNase specific activity ratios (inoculated: healthy) of the two lines of wheat at different times after inoculation and incubation at 20° C and 26° C. The specific activity of the RNase from healthy plants varies only slightly during the experimental period, so that an increase in the ratio is the result of an increase in the specific activity of RNase in the inoculated leaves. The results in Fig. 1 indicate that the characteristic bimodal increase in RNase activity occurs only in host-parasite combinations that result in susceptible (type 3 to 4) reactions (*sr6*-race 56 and *Sr6*-race 56A at 20° C; *Sr6*-race 56 and *Sr6*-race 126 ANZ-6, 7 at 26° C). In combinations that result in resistant (type 0 or 0;) reactions (*Sr6*-race 56 and *Sr6*-race 126 ANZ-6, 7 at 20°C) only the early phase of RNase increase occurs. These results show a positive correlation between changes in the levels of RNase and the continued growth of the rust in host tissue.

Figure 2 shows the development of late RNase in groups of *Sr6* plants transferred from 20 to 26° C at various times after inoculation with race 56. Plants transferred 12 h (Fig. 2*a*) after inoculation show the normal pattern of early and late RNase activity exhibited by plants inoculated and maintained at 26° C (Fig. 1*c*). In plants maintained at 20° C for 24 h (Fig. 2*b*), 5 d (Fig. 2*c*), and 13 d (Fig 2*d*) before transfer to 26° C, the appearance of late RNase activity is increasingly delayed; in plants maintained continuously at 20° C, no late RNase activity develops (Fig. 2*e* and Fig. 1*a*). In addition, the initial appearance of sporulating rust pustules (indicated by inverted arrows in Fig. 2) is increasingly delayed and their number is progressively reduced. In Fig. 2, the number of pustules in each treatment is: *a*, 15 to 20; *b*, 12 to 15; *c*, 5 to 7; *d*, 1 to 2; and *E*, zero. In each case, when pustules first appear, regardless of their frequency and time of appearance, the value of the RNase specific activity ratio is between 2.4 and 2.7 (Fig. 2). These results show that the appearance of late RNase accompanies the resumption of the growth of the pathogen, but that the quantitative increase in RNase is not a function of the amount of rust mycelium present in the infected leaf.

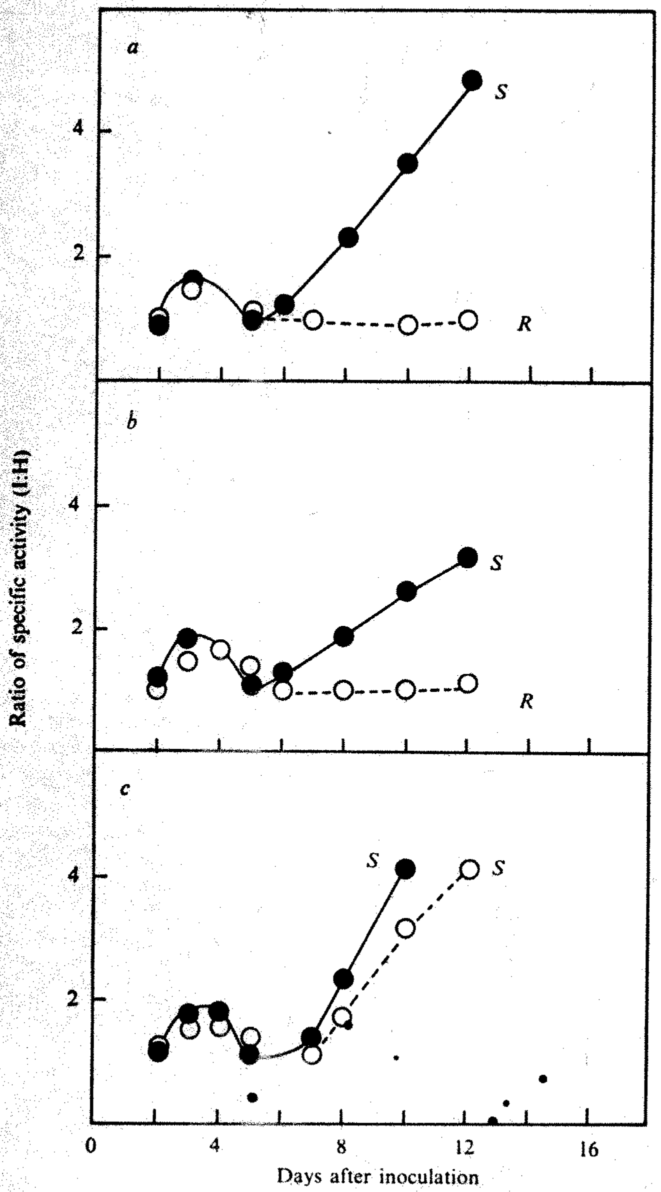

FIG. 1 The effect of temperature regimes on quantitative changes in the RNase activity of the near-isogenic lines of wheat carrying the *Sr6* and the *sr6* alleles following inoculation with wheat rust races 56, 56A and 126 ANZ-6,7. RNase was extracted from healthy and inoculated primary leaves at 0 to 4° C. Approximately 5 g of leaves were ground in a prechilled mortar with equal amounts (on a weight basis) of sand and polyvinylpolypyrrolidone (Sigma Chemical Co., St. Louis, Missouri). This was extracted with 15 ml of 40 mM potassium phosphate buffer, *p*H 6.7, and centrifuged at 10,000*g* for 30 min. The supernatant fraction was acidified to *p*H 5.0 with 1 N HCl and left at 0 to 4° C overnight. The precipitate formed during this interval was removed by centrifugation at 10,000*g* for 15 min. The supernatant was used for the estimation of RNase activity. The reaction mixture for RNase assay contained (final volume 1 ml) sodium acetate buffer, *p*H 5.0, 50 μmole; yeast RNA (high molecular weight), 0.8 mg and RNase (15 to 25 μg protein). Duplicate samples were run without the enzyme. All samples were incubated at 37°C for 30 min, chilled quickly and 2 ml of precipitating reagent (1N HCl in 76% ethanol containing 0.5% $LaCl_3$) was added to each sample. The content of the tubes was mixed thoroughly, allowed to stand for 10 min and centrifuged until the supernatant was clear. The A_{260} of the supernatant was read against similarly diluted precipitating reagent and corrections were made for the enzyme blanks. One unit of RNase is defined as the quantity of enzyme catalyzing an increase in A_{260} of 1.0 in the standard conditions of assay. Specific activity is units per mg protein. *S*, Susceptible; *R*, resistant. *a*, 20°C; ●, *sr6*-race 56; ○, *Sr6*-race 56. *b*, 20°C; ●, *Sr6*-race 56A; ○, *Sr6*-race 126-ANZ-6, 7. *c*, 26°C; ●, *Sr6*-race 56; ○, *Sr6*-race 126-ANZ-6, 7.

Table 1 The substrate preference of RNases from healthy and rust-infected *Sr6* and from axenically-grown rust mycelium.

Source of RNase	Growth temperature	Relative rates of hydrolysis (d.p.m. solubilised per mg protein × 10^{-4}) Poly (A)	Poly (U)	Poly (C)
Sr6 (Healthy)	26°C	130	195	75
Sr6 (10 ds after inoculation with race 126 ANZ-6, 7)	26°C	289	360	430
Rust mycelium (race 126 ANZ-6, 7)	17°C	267	200	265

Primary cultures of the Australian isolate of wheat rust race 126 ANZ-6, 7 were grown on a chemically defined liquid medium containing, the following (in g l^{-1}): glucose, 3.0; $NaNO_3$, 2; KH_2PO_4, 1; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $FeSO_4.\ 7H_2O$, 0.01; glutamine, 6.1; cysteine, 0.36; $Ca(NO_3)_24H_2O$, 2 and sodium citrate, 1.5 at *p*H 5.5. About 2 g of rust mycelium was extracted as described in the legend of Fig. 1. The homopolymers of adenylic, uridylic and cytidylic acids (potassium salt, specific activity 20 μCi/μmol P) were purchased from Miles Laboratories, Inc., Elkhart, Indiana). The reaction mixtures for the radioassay were the same as those described in the legend to Fig. 1 except that the ^3H-labelled homoribopolymers were used instead of yeast RNA. At the end of the incubation period, 0.3 ml of non-radioactive yeast RNA (3.0 mg ml^{-1}) was added to each tube as a coprecipitant and mixed thoroughly before the addition of the precipitating reagent. After centrifugation, an aliquot of the supernatant was processed[9] for the estimation of radioactivity in a Nuclear Chicago (Mark I) liquid scintillation spectrometer. The c.p.m. of the samples were obtained by the channels ratio counting procedure and the efficiency of counting was determined using a quench correction curve prepared with standard ^3H-labelled samples. The radioactivity (d.p.m.) was calculated from these data.

The results in Table 1 show that the RNase from rust infected wheat leaves hydrolyses poly(A), poly(U) and poly(C) at rates 2.2, 1.8 and 5.7 fold those of the enzyme from corresponding uninfected leaves. In addition the substrate preferences of the RNase from uninfected leaves, infected leaves and rust mycelium grown in axenic culture are all different and are, poly(U) > poly(A) > poly(C); poly (C) > poly(U) > poly(A) and poly (A) > poly(C) > poly(U) respectively. Similar changes in the substrate preference of the RNase in flax cotyledons inoculated with *Melampsora lini* and in *Ribes* leaves and cortical tissue of pine inoculated with the diploid and haploid phases of *Cronartium ribicola*, respectively, have been reported elsewhere (ref. 1 and A. E. Harvey, A. K. C., M. S., and L. A. S., unpublished). The results presented thus far show that there is a direct correlation between the ability of the rust fungus to grow in host tissue and the appearance of late RNase (Figs 1 and 2), which has a substrate preference different from the RNases of uninfected host tissue and rust mycelium grown in axenic culture. They suggest that RNase molecules with unique catalytic properties may be required for growth of the pathogen in the host. If this idea has any validity it should, in principle, be possible to detect changes in the properties of RNase in the initial stages of growth of the pathogen in the host.

A series of experiments was therefore carried out in order to investigate this possibility (Table 2). Healthy and inoculated (race 56) *Sr6* plants were maintained at 20° C for 13 d (Fig. 2*d*). In these conditions, growth of the rust is initiated but is restricted to a few intercellular hyphae, as described earlier. On the fourteenth day, batches of healthy and inoculated plants were transferred to 26° C. Changes in RNase levels, heat stability and substrate specificities were measured at 3, 6, 10, 13 and 21 d after inoculation (that is 7 d after transfer from 20° C to 26° C. Table 2). For comparison, similar measurements were also made using plants maintained continuously at 26° C for 10 d after inoculation (Table 2). As expected, late RNase does not appear until after the plants are transferred to 26° C. The heat stability (at 80° C for 10 min; see legend to Table 2) of the RNase from healthy leaves varies between 60 and 70%, regardless of the temperature at which the plants are held. The stability of the enzyme from infected tissue is consistently lower, and varies from 52% 3 d after inoculation to 31% at 13 d in plants held at 20° C (Table 2). When late RNase appears at 26° C, its heat stability is even lower (22 to 23%, Table 2). Changes in the heat stability of RNase thus precede the appearance of late RNase.

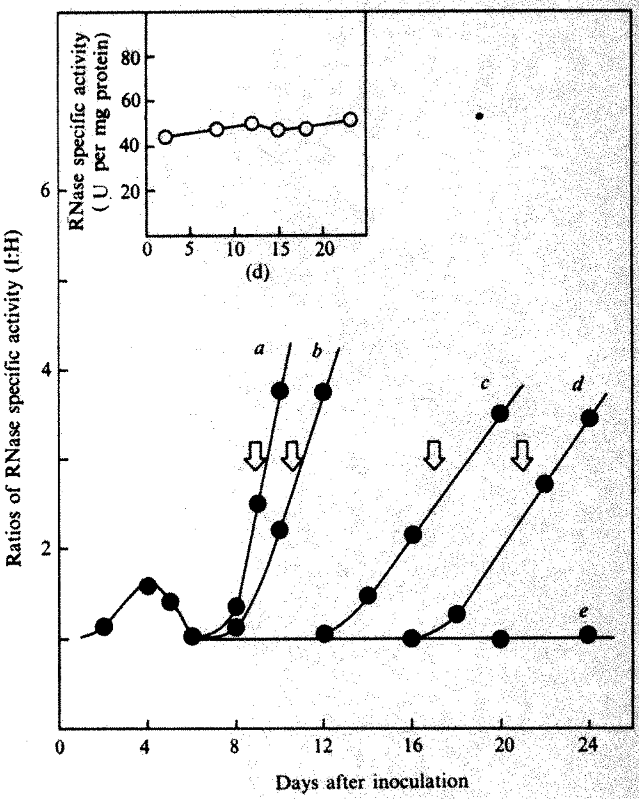

Fig. 2 Changes in the RNase specific activity of wheat plants carrying the *Sr6* allele inoculated with wheat rust race 56 and transferred to 26° C at various times after inoculation. *a-e,* Five groups of healthy and inoculated plants were maintained at 20° C for different times before they were transferred to 26° C. The plants of group *a* were transferred 12 h after inoculation and those of *b*, *c* and *d* at 24 h, 5 d and 13 d after inoculation respectively. *e*, Healthy and inoculated plants never transferred to 26° C. Arrows indicate the approximate time when pustules become visible. The inset shows the specific activities (units per mg protein) of extractable RNase of the primary leaves of healthy plants of group, *d*, at various intervals beginning with the time when other plants of the same group were inoculated.

The relative rates of hydrolysis of poly(A), poly(U), and poly(C) also show striking changes as early as 3 d after inoculation in plants held at 20° C (Table 2). Poly(C) hydrolysing activity increases 3 fold on the third day after

Table 2 Effect of temperature regimes on *a*, quantitative and qualitative changes in RNase activity and *b*, intensity of infection in the wheat variety carrying the *Sr6* allele following inoculation with wheat rust race 56.)

		Sr6 race 56, at 20° C until day 14, then transferred to 26° C					*Sr6* race 56 at 26° C
Days after Inoculation		3	6	10	13	21	10
1 RNase specific activity ratios (I:H)		1.50 (Early RNase)	1.08 (Early RNase disappearing)	0.96 (No late RNase)	1.02 (No late RNase)	2.30 (Late RNase)	3.7 (Late RNase)
2 Heat stability (% of original activity retained, 10 min at 80° C).	H	63	70	61	64	61	60
	Inoc	52	42	32	31	22	23
3 Relative rates of hydrolysis (Inoc/H) of:							
Poly (A)		1.26	1.49	1.46	1.85	3.70	2.60
Poly (U)		1.21	0.80	0.90	1.07	3.60	2.10
Poly (C)		3.16	1.27	1.02	1.04	4.10	3.90
4 Intensity of infection		No visible symptoms	No visible symptoms	No visible symptoms	No visible symptoms	1 or 2 small pustules per leaf	15–20 postules per leaf

The primary leaves of *Sr6* wheat were inoculated with wheat rust uredospores (race 56) and maintained at 20° C. 14 d after inoculation, batches of healthy and inoculated plants were transferred to 26° C. 'Early RNase' and 'late RNase' refer to the early and the later phases of increase in the RNase specific activity ratios (inoculated:healthy) (I:H)) respectively, as shown in Fig. 1. Heat stability of RNase was estimated by the following procedure[1]. The *p*H of the RNase solution was raised to 8.5 by adding 1 M NaOH and an aliquot of the enzyme was held at 80° C for 10 min, cooled to 0° C, the *p*H readjusted to 5.0 with 1 N HCl. The activity of the enzyme was estimated and the loss of activity due to heating at 80° C was determined. The results are presented as the percentage of original activity retained at 80° C. The relative rates of hydrolysis of ^{3}H-labelled poly(A), poly(U) and poly(C) were determined as described in Table 1. The ratios of the rate of hydrolysis (d.p.m. solubilised per mg protein) of the RNase from inoculated leaves to that of the enzyme from healthy leaves for each polynucleotide are presented.

inoculation. Poly(A) hydrolysing activity increases by 26, 49, 46 and 85% on the third, sixth, tenth and thirteenth days after inoculation; after transfer to 26° C and the appearance of late RNase 21 d after inoculation, it increases by more than 3 fold (Table 2). The changes in poly(C) and poly(U) hydrolysing activities appear to parallel total RNase activity; they increase when early RNase appears, decrease when it declines and increase again by 3 to 4 fold after the plants are transferred to 26° C and late RNase appears. These results show that in infected leaves, changes in heat stability and substrate preference of the enzyme precede the increase in activity that we have called late RNase.

Sporulating pustules developed at only one or two infection sites per leaf on the *Sr6* plants held first at 20° C for 13 d and then at 26° C for 7 d. On the other hand, when *Sr6* plants were held at 26° C for 10 d immediately following inoculation, fifteen to twenty type 3 to 4 pustules developed on each leaf. Comparison of the decreases in thermal stability and increases in the rates of polynucleotide hydrolysis by the late RNase from these two samples (Table 2), strongly indicates that the changes in the properties of the enzyme are not directly related to the number and size of the sporulating pustules that finally develop. It is, however, important to remember that each leaf was inoculated with uredospores over its entire surface so that the number of incipient infections is high (at least fifteen to twenty) in each case.

The results presented here show that infection of wheat with wheat stem rust is accompanied by an increase in early RNase, which reaches its maximum activity at 3 or 4 d after inoculation and then declines. In susceptible combinations, the decline in early RNase is followed by a marked rise in late RNase. The appearance of late RNase is correlated with the ability of the pathogen to grow in the host tissue, but not with the number and size of the pustules that finally develop. Increases in the activity of both early and late RNases are characterised by major differences between the heat stabilities and substrate preferences of these enzymes and those of the RNases of the uninfected host and rust mycelium grown in axenic culture. Such qualitative changes in RNase activity are detectable within 3 d after inoculation. In principle they may well be initiated in the very first stages of host-parasite interaction and may play essential roles in the post transcriptional processing of RNA during the host parasite interactions in both susceptible and resistant combinations. This suggestion is supported by the observation that in rust infected wheat[7] and flax[8] increases in RNase activity are accompanied by increased RNA synthesis, which in a rust-infected susceptible variety of flax, involves the preferential synthesis of one or more molecular species of RNA with a high A + U/G + C ratio[8].

We thank Mr A. Bose for providing the axenic cultures of wheat rust and Dr J. M. Daly, University of Nebraska, for a gift of seed of the near-isogenic lines of wheat. This work was supported by grants (to M.S.) from the National Research Council of Canada and the Canada Department of Agriculture.

Note added in proof: The unpublished paper by A. K. Chakravorty, M. Shaw and L. A. Scrubb and A. E. Harvey, A. K. Chakravorty, M. Shaw and L. A. Scrubb are now 'in the press' *Physiological Plant Pathology*.

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book reviews

Medicine in China

Medicine and Public Health in the People's Republic of China. Edited by Joseph R. Quinn. Pp. xii+333. US Department of Health, Education and Welfare, National Institutes of Health: Bethesda, Maryland, 1973.)

THIS is not a very satisfactory book on three grounds. First, it suffers from the usual disadvantage of a multi-authored book in which no editorial attempt has been made to avoid redundancies and overlap. Second, although the title implies an up-to-date coverage of the subject, this is really only true of two or three out of the fourteen chapters. In fact, of the fourteen authors only two (Dr and Mrs Seidel) wrote out of personal knowledge based on recent visits to the People's Republic of China (PRC). At the time of publication (June 1972 for preliminary soft cover edition) none of the other authors seemed to have visited the PRC during the preceding two decades. Third, it is simply not clear for whom this book was envisaged in spite of the introductory statement that "inasmuch as the audience for this document is intended to be those persons with a general interest in the subject of China medicine rather than the biomedical specialist, the lack of confirming evidence by on-site observation or experimentation was not considered a deterrent to the preparation of this document". This may not have been a deterrent for the authors, but it is likely to deter many readers.

I myself had been unfamiliar with the state of medicine and public health in the PRC prior to a lecture visit in 1973. In preparation for that visit I had skimmed over this book, but had read carefully only two chapters ("Pharmacology" by J. Y. P. Chen and "Population Dynamics" by L. A. Orleans) because they were directly related to my own interests. Only Orleans's chapter proved to be instructive. Upon my return, I reread carefully the entire book and reached the conclusions that as a primer for prospective visitors to the PRC, no more than a third of it is relevant to the present scene and reasonably up to date. The rest is history and much of it so superficial that its main utility is the list of references at the end of most chapters. Specific mention of a few chapters will suffice to illustrate the pros and cons of this book.

R. C. Croizier's chapter on "Traditional Medicine as a Basis for Chinese Medical Practice" offers a brief but reasonably good background to why traditional Chinese medicine with its theoretical basis should be distinguished from ordinary folk medicine. Croizier also explains why this was retained by the Communist regime and integrated into their current medical system together with Western medicine: "After all, if an engineer could learn from a coolie, and an agronomist from an old peasant, why not have a modern medical specialist learn from a native herbalist?" The brief description of the dual forces of the universe, *yin* and *yang*, together with the vital life force, *ch'i*,—though necessary to an understanding of traditional Chinese medicine—is repeated in three other chapters. Even a minimal editorial effort could have avoided some of this duplication. Perhaps the most appropriate place for a discussion of this philosophical basis of Chinese medicine is the longest chapter of the book, "Acupuncture" by J. Y. P. Chen. Although its length is appropriate in view of the importance of acupuncture in China, both past and present, it overdoes the detailed operational aspects at the expense of an adequate discussion of its theoretical basis and of current work on the mode of action of acupuncture anaesthesia.

The other chapter by Chen entitled "Pharmacology" is based primarily on a perusal of Chinese journals published until 1967 and subsequently upon newspaper accounts. Its list of plant and animal drugs ranges from the superficial to the ridiculous (for example, grasshoppers and locusts "have the effect of calming the nerves and stopping coughs and they are used in whooping coughs and tetanus. Grasshoppers are said to be specific for cases of pertussis and asthma"). By contrast, China's modern pharmaceutical industry rates only one page. Yet this is one of the most impressive achievements of the PRC. Few Western visitors are prepared for the total self sufficiency of the PRC in terms of modern drugs—ranging from all currently used antibiotics to steroid oral contraceptives which are synthesised on a scale which is hardly equalled in the United States. No mention is made of the first class work on the isolation and chemical characterisation of traditional Chinese herbal medicines at the Institute of Materia Medica in Peking. Nowhere in this chapter, or indeed anywhere else in this book is there any description of the rather impressive labelling practice in the PRC—each drug container listing the date of manufacture, name of factory, name (in Latin for "Western Medicines") of drug, quantitative analysis, and so on. In fact, V. Seidel's chapter on "Medical Personnel and their Training" with his table (pages 169–171) listing the contents of a "barefoot doctor's" bag is much more instructive in giving the reader a view of the state of today's drug development in the PRC.

By far the most readable chapter in the entire book is the one on "Population Dynamics" by L. A. Orleans, which deals in an authoritative way with demographic aspects, family planning programmes and public health. What is not clear to the uninitiated reader is that it reflects accurately the state of affairs up to the Cultural Revolution, but that much has changed in the PRC during the past five years in the field of birth control (see C. Djerassi, *China Quarterly*, **57**; 1974). Curiously enough, this best chapter is the only one which lacks references.

The price of this book is not indicated but is likely to be relatively cheap since it is published by the US Department of Health, Education and Welfare. Nevertheless I am hard pressed to guess for whose library it should be purchased.

CARL DJERASSI

Rare earth magnets

Rare Earth Intermetallics. By W. E. Wallace. (Materials Science and Technology.) Pp. xii+266. (Academic, (Harcourt Brace Jovanovich): New York and London, September 1973.) $22.50.

THE extraordinary magnetic properties of the rare earth metals—which are not really rare—have only been appreciated during recent years, and the time is ripe for a concise appraisal. Herein lies the great value of this monograph which provides a fascinating, orderly and critical presentation of the properties of rare earth intermetallic compounds, collated up to the summer of 1971.

The authors give a useful, short table of symbols to describe the main types of magnetic order and paramagnetic behaviour. They discuss types of magnetic interaction and the Rudermann-Kittel-Kasuya-Yoshida concept of indirect exchange interaction between rare earth ions through conduction electrons, and the de Gennes contributions to the theory. A chapter on (electrostatic) field interaction and its effect on magnetic and thermal behaviour of rare compounds pays tribute to the pioneer work of Penney and Schlapp, Bleaney and Stevens, and Van Vleck which helps us to explain so much of this work. A helpful illustration is given of the use of the Stevens operator equivalent method of dealing with the magnetic and thermal effects of the crystalline field on rare earth ions, but it is difficult to understand the necessity or wisdom of including in this book an appendix of forty-eight very closely packed pages of numerical data on energies, eigenfunctions, and so on of rare earth ions in a hexagonal field.

I much appreciated the orderly layout of the five chapters on rare earth-non-transition metal systems, and of the four chapters on rare earth-transition metal systems in which Ni, Co, Fe and Mn loom large, each with its wealth of tables of magnetic characteristics and graphs of magnetic and thermal behaviour. I was also interested in the many references to the Kondo effect, and to Mössbauer and neutron diffraction experiments. Much discussion is given to phenomena which are associated with the filling of the *d* bands in the transition metals—the variation of the magnetic moment of cobalt and iron with temperature. A brief treatment is given of the use of rare earth materials for permanent magnets, where the rare earth is thought to provide a uniaxial crystal with high Curie point, large saturation magnetisation and strong anisotropy. The commercial evaluation of the RCo_5 system is in its infancy; the RFe systems do not seem encouraging, but perhaps RFeCo systems may later be found to be so.

This is an excellent monograph; though it would be improved by the addition of a subject index.

L. F. BATES

Inorganic initiation

Inorganic Chemistry: Principles of Structure and Reactivity. By James E. Huheey. Pp. xvi+737. (Harper and Row: New York, Evanston, San Francisco and London, 1972.) £3.85.

THIS is an interesting, original and stimulating book but a difficult one to place: it certainly cannot be regarded as a comprehensive text on inorganic chemistry. Its main aim seems to be to introduce the university student to the fascination of research publications in many areas of modern inorganic chemistry: it starts typically with perbromate on page 1 and xenon tetroxide on page 2.

The first two thirds of the book are concerned with the development and application of principles, the emphasis throughout being on structure and bonding in molecular compounds and complexes. Solid state chemistry and thermodynamics are virtually absent, but there is a very full discussion of the many attempts to rationalise covalent bonding in inorganic systems. The last third is a series of essays on such topics as organometallic chemistry; chains, rings and cages; noble-gas and positive-halogen chemistry; lanthanides and actinides; and biological systems.

The book is very easy to read, full of delightful touches, and clearly the work of an enthusiastic teacher. It is well illustrated and has excellent references to further readings on a wide variety of subjects. It will surely appeal to many students and it is excellent value.

C. S. G. PHILLIPS

This earthly life

Life on Earth. By Edward O. Wilson *et al.* Pp. xi + 1033. (Sinauer: Stamford, Conn; Freeman: Reading, September 1973.) £5.40.

THIS college biology textbook claims to "pioneer a new generation" of such publications. If it is not, perhaps, such a complete innovation as this, it is, nevertheless, a good basic book which has some unique features.

A publication which boasts eight authors runs the risk of presenting a divisive view of its subject, but the quintessence of modern biology offered here escapes this hazard. Although the sections differ in style and approach, these differences merely reflect the intrinsic differences in their themes and a sound system of indexing and cross reference helps to unify the whole.

It starts with a kind of prologue, "Life on the Third Planet". There is an opening quotation from James A. Lovell who saw the earth, as he returned to it in Apollo 8, as "the only place that had color, the only place that had life . . . that had warmth . . . that was home to us". The first of the five main sections, "The Cell", covers, in about one quarter of the book, the structure of the cell, biological reactions, enzymes, energy, codes and genes, genetics and the control of cell chemistry. Section 2, "Multicellular Life", again comprises about one quarter of the whole and includes chapters on: development, reproduction, carbon and nitrogen sources, gas exchange, transport systems, the internal environment, neurobiology, effectors, behaviour instinct and ethology, and the modification of behaviour. Section 3 describes the diversity of life in a traditional way and Section 4, "The Strategy of Evolution", includes chapters on biogeography, the growth and interaction of populations, ecosystems, social behaviour and human evolution. The last section, "Alternate Futures", presents contrasting optimistic and pessimistic prophecies.

In all, this is a comprehensive, exciting and intellectually challenging book which throws illuminating sidelights on the personalities and work of biologists, on the use of biological materials in everyday concerns and on the significance of biological processes for mankind. The format is pleasing, if fragmentary, there are many striking photographs, and explanatory diagrams are well correlated with the text. Controversial issues are developed in parenthetic, colour-panel insertions so that they do not interrupt the development of the main thesis. A list of related readings from specialised books, articles and research papers is given at the end of each chapter. There is a glossary of biological terms. *Life on Earth* would certainly be a useful volume in a school or college library and might serve as a basic, if weighty, sixth form or college text.

E. J. VINNICOMBE

Interface chemistry

An Introduction to the Principles of Surface Chemistry. Edited by R. Aveyard and D. A. Haydon. (Cambridge Chemistry Texts.) Pp. xvi+232. (Cambridge University: London, August 1973.) £5.40 cloth; £2.40 paper.

THIS book is a must for all surface chemists, but particularly those meeting the subject for the first time, either in formal course work or starting research. I also strongly recommend it for all those teaching the subject as it is one of the clearest and most logical expositions of the physical chemistry of interfaces I have encountered. It assumes the reader is familar with the basic thermodynamics and statistical mechanics met in most undergraduate courses, and on this foundation builds the subject up to a level where the reader can take the subject further by reading some of the heavier texts or recent review articles referred to in the book. It is refreshing to see a limited number of carefully selected, relevant references referred to in the text, rather than the seemingly endless list, bewildering to the beginner, normally encountered.

The authors do not exceed the aim set in the book's title. It does not attempt to deal in depth with experimental aspects, or applications, of the subject, thus leaving room (unlike many other text books in the field) to give full derivations of many of the more important equations. In short, this book fills a gap in the literature and is well worth the price.

B. VINCENT

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- What is the twin paradox?
- Why has the Earth not cooled down?

This kind of programme should be compulsory listening for all producers of science programmes. There clearly is a broad interest in science, which BBC Radio is still not catering for satisfactorily. In particular, perhaps it is not too late for "Kaleidoscope" to live up to its description as Radio 4's nightly review of the arts and sciences. The only thing wrong with the science on "Kaleidoscope" is that often one feels the need of a microscope to find any; perhaps the recent arrival of "Scientifically Speaking" (see *Nature* **247**, 411; 1974 for a review) will be taken as a spur to show that, at least in the 'science is fun' department, "Kaleidoscope" can do for the layman what John Maddox seems to be trying to do for the professional scientist.

Welcome surprise

David Davies

Independent Television served up an unexpected offering on January 31—half an hour on anorexia nervosa—and on this showing they should chance their arm more often on science and medicine. Certainly there are enough diseases to keep British viewers fascinated for a whole season.

The programme was written, produced and directed by Robin Brown and it was a brave job, although one suspects he could have done with a bigger budget. As it was the presentation fell between two stools. The disease, a compulsive teenage refusal to eat adequately, was clearly described early on, and this would have made an excellent fifteen minute documentary, one of a series. But Mr Brown's imagination, or pocket, did not stretch to half an hour and so things got thinner as time went on. With such a length of programme it is necessary to go into some detail—is it a complaint of the well-off only, how many actually die of it, how many later lead a perfectly normal life, how does it divide amongst the sexes? One hardly got the impression that it had been difficult cramming everything in.

Moreover, there was a strange air of unreality to many of the scenes. If viewers can take starving Ethiopians, they can presumably also take over-slim English girls, yet none of those shown looked in any great distress; in fact they all seemed to have got better before being filmed. Two minutes with someone visibly suffering would have got the story over very effectively. This lack of verisimilitude was compounded by stilted dialogue.

There was, however, one quite extraordinary moment. Anorexia nervosa is a complaint which tends to involve and confuse the family and generate strange family situations in which powerful forces are abroad but are manifested in odd material ways. A mother was filmed reminiscing with her daughter about spraying wash basins and lavatory bowls with red ink to attempt to dissuade her from being sick in them and having to wash away the tell-tale markers. This was a stunning insight into the dilemma of the family and produced a chilly moment.

We were mercifully spared commercials till the end when we were exhorted to consume baby foods, thick soups and juicy oranges.

obituary

A. S. Romer

Dr Alfred Sherwood Romer, Emeritus Professor at Harvard University, who died on November 5, aged 78, became one of the most outstanding names in the history of Vertebrate Palaeontology. Graduating at Amherst he moved to Columbia University where he worked in the school of Professor W. K. Gregory and was first concerned with the leg musculature of dinosaurs. In 1923 he was called to the University of Chicago and here his major work was on the Pelycosauria. After many years of field work and laboratory study, and after publishing a series of papers, he wrote with L. I. Price, a brilliant artist, a monograph on these animals published in 1940, which is a model for such work. He also published valuable papers on other early reptiles and the amphibia, and he wrote a monograph on the labyrinthodonts in 1947 which will long remain the standard work on these animals.

In 1937 Romer sectioned the head of the fish *Ectosteorhachis* and, as a result, he immediately recognised the correctness of Westoll's solution to the problem of fish dermal bone nomenclature. He was a leading champion of the view that vetebrates evolved in fresh water. This reflects the constant attention he paid to the geological background of the animals he studied, work which resulted in a sound knowledge of the continental deposits of the Lower Permian of Texas on which he became a recognised authority.

Romer discussed usefully such troublesome matters as the nature of *Diadectes*, the origin of the Ichthyosauria, and the relationships of *Araeoscelis* and of the so-called Microsauria. It was Romer who realised that the phyllospondylous amphibia were not an independant order, as had long been held, but only the larvae of labyrinthodonts as was originally believed. He questioned the view that the rhachitomous vertebrae of labyrinthodonts were derived from the embolomerous type and was eventually able to demonstrate that the reverse was, in fact, true.

At an age when most palaeontologists have long ceased from active field work

Al Romer was making discoveries of new faunas in South America. This entailed hard physical exertions and resulted in the discovery of extremely important Middle Triassic faunas (missing from the classical South African Karroo) including both synapsid reptiles, long needed to illustrate fully the origin of mammals, and of new early archosaurs. These explorations resulted in a series of clearly written papers, showing the balanced judgement which characterised Romer's writings throughout his long working life.

One of Romer's greatest calls to fame lies in his text books. His *Vertebrate Palaeontology*, published in 1933 when no other such work existed in the English language, was an immediate success. It surveyed the whole field of vertebrate palaeontology with balance and judgement and was excellently illustrated and it was of the greatest help to all students of the subject. The accompanying *Man and the Vertebrates*, of a lesser standard, was intended to protect the publishers against a loss on the main work—which never occurred. His famous book was republished in 1945 and was almost entirely rewritten and reillustrated again by the author in 1966, thirty three years after the publication of the first edition! He also published in 1949 a book on the comparative anatomy of the vertebrates, called *The Vertebrate Body*, of great value to students, dealing with the embryology, osteology and dentitions (he once remarked "I hate teeth"), the circulatory system, the CNS, the myology and the digestive systems. Not content with that he published in 1956 his famous *Osteology of the Reptiles*, to replace the largely out of date work by Williston. Here, as in his *Vertebrate Palaeontology*, his name will long remain a familiar one on the shelves of zoological libraries. His last book, *The Procession of Life*, was published in English in 1968; it was published also in French, German and Italian and these Continental editions are superbly illustrated with many coloured plates.

Romer became Director of the Museum of Zoology at Harvard in 1946, having been Professor of Zoology and Curator of Vertebrate Palaeontology since 1934. He was Alexander Agassiz Professor of Zoology from 1947 until 1965 and was then elected Emeritus Professor. He was held in the greatest respect all around the world, which he travelled extensively, and was honoured by the award of an immense number of medals and honorary degrees. He was a Member of the Academy of Sciences, a Past President of the American Association for the Advancement of Science a Foreign Member of the Royal Society and an honorary member of many others.

When his research work, his teaching and his books are considered together his contribution to vertebrate zoology can only be described as formidable.

A host of friends were indebted to him and his charming wife for their never failing help, kindness and hospitality.

Announcements

Appointments

P. C. Elwood has been appointed **Director** of the **Medical Research Council Epidemiology Unit** (South Wales).

Professor **J. Heslop-Harrison,** FRS, Director of the Royal Botanic Gardens, Kew, has been elected **President** of the **Institute of Biology** for 1974.

Professor **J. H. Beynon,** senior research associate at ICI and professor of chemistry at Purdue ~~University~~, Lafayette, has been appointed to a **Royal Society Research Professorship** at the **University College of Swansea, University of Wales.**

Sir Owen Saunders, FRS, Emeritus Professor of Mechanical Engineering at Imperial College, University of London, has been appointed **Royal Society Visiting Professor** at the **Engineering School** of the **Federal University of Rio de Janeiro.**

Awards

The first **Tribology Bronze Medals** of the **Institution of Mechanical Engineers** have been awarded to **D. R. Adams, D. R. Garner** and **R. A. Onions.**

Aslib (formerly the Association of Special Libraries and Information Bureaux) is to mark its 50th anniversary in 1974 by the award of a prize of £250 and a piece of engraved silver for the best paper submitted in competition on *The Future Pattern of Information Services for Industry and Commerce.*

International Meetings

March 1, **Biodegradation of Town Refuse and Farm Wastes** (Marian Honer, Assistant Information Officer, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET)

March 4, **Catalysis and Inhibition of Oxidation Processes** (Dr R. F. Greenwood, Hon. Secretary, London Section, Society of Chemical Industry, Chemistry Department, The City University, St John Street, London EC 1V 4PB)

March 4–6, **Interim Meeting of the Society of Clinical Pathologists and the College of American Pathologists** (Steven K. Herlitz, Inc., 850 Third Avenue, New York, N.Y. 10022)

March 4–8, **25th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy** (Robert W. Baudoux, Exposition Chairman, U.S. Steel Corporation, Research Laboratory, M.S. 57, Monroeville, Pennsylvania 15146)

March 5–6, **Noise Measurement and Control** (Course Organiser, Birniehill Institute, National Engineering Laboratory, East Kilbride, Glasgow G75 OQU)

March 6, **Meeting of the Particle Size Analysis Group of the Society for Analytical Chemistry** (Analytical Division, Chemical Society 9/10 Savile Row, London W1X 1AF)

March 6–8, **Minicomputers: Fundamentals and Applications** (Lisa Spaducci, Polytechnic of Central London, 115, New Cavendish Street, London W1M 8JS)

March 7, **Component Interactions in Fluid Flow Systems** (A. J. Tugwell, 1, Birdcage Walk, Westminster, London, SW1H 9JJ)

March 11–15, **Chemical Society Management Studies for Chemists** (Dr M. D. Robinson, The Chemical Society, Burlington House, London W1V OBN)

March 13, **Prediction of Geological Hazards** (Professor B. M. Funell, Environmental Sciences, University of East Anglia, Norwich NOR 88C)

March 14–15, **Heat Pipes** (The Short Course Unit, The Polytechnic of Central London, 35, Marylebone Road, London NW1 5LS)

March 18, **Microcalorimetry in Biology** (The Assistant Secretary, Society of Chemical Industry, 14, Belgrave Square London SW1X 8PS)

March 18–22, **6th Semi-Annual Short Course on Laser Safety** (Laser Safety Course, CONMED, 114 Medical College, Cincinnati, Ohio 45229)

March 19, **Group Technology: A Method for Improving Productivity** (Course Organiser, Birniehill Institute, National Engineering Laboratory, East Kilbride, Glasgow G75 OQU)

March 21, **General Meeting of the Institution of Mining and Metallurgy** (The Geological Society, Burlington House, Piccadilly, London W1V OJU)

March 20–21, **Computers in Medicine** (The Secretary, World Organisation of General Systems and Cybernetics, Blackburn College of Technology and Design, Feilden Street, Blackburn BB2 1LH)

March 20–22, **Tetrahedrally Bonded Amorphous Semiconductors** (Marc H. Brodsky, Conference Chairman, IBM Thomas J. Watson Research Center P.O. Box 218, Yorktown Heights, New York 10598)

Classified Advertisements

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APPOINTMENTS VACANT

University of Leeds

DEPARTMENT OF PLANT SCIENCES

Applications are invited for the post of DEMONSTRATOR IN SOIL PHYSICS. Candidates should have a good first degree in soil science or an allied discipline, and have an interest in soil physics and physical chemistry as applied to the growth of crops in the field. An interest in computer modelling would be an advantage. Salary within the range £1,290 to £1,971.

Forms of application and further particulars from the Registrar, The University, Leeds LS2 9JT (please quote 41A/1D). Closing date March 12, 1974. (732)

UNIVERSITY OF GLASGOW

DEPARTMENT OF BIOCHEMISTRY

RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant in the Department of Biochemistry. The person appointed will work on the control of uterine development and the molecular mechanism of steroid hormone action.

The salary will be within the range £1,400 to £1,550 or £1,600 to £1,800 per annum depending upon qualifications and experience. F.S.S.U.

The post is available immediately and is tenable for 1 year in the first instance.

Applications should be lodged not later than February 28, 1974, with Dr R. E. Leake, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, from whom further particulars may be obtained. In reply please quote Ref. No. 3411M. (754)

NORTH STAFFORDSHIRE HOSPITAL CENTRE

RESEARCH ASSOCIATE (JUNIOR RESEARCH FELLOW) to study prostaglandin activity in alimentary disorders under Dr. C. H. J. Swan, Consultant Gastroenterologist. Candidates should have an appropriate first degree, and facilities may be available for a suitable candidate to register for a higher degree in the University of Keele.

Starting salary: £1,398 per annum.

Applications to: Mr. W. J. Cowen, Administrative Officer, Central Outpatient Department, Hartshill Road, Stoke-on-Trent, Staffordshire. Telephone: 0782-44161, ext. 4202, from whom further information may be obtained.

Closing date for applications: March 14, 1974. (776)

MARINE BIOLOGICAL ASSOCIATION

A SCIENTIFIC ASSISTANT is required at the Plymouth Laboratory to work in the Electron Microscope section. Minimum qualifications are four G.C.E. "O" level passes including Chemistry or another scientific subject, English or English Language or a Mathematical subject. A knowledge of photography would be an advantage. The appointment is in the Assistant Scientific Officer grade. Salary according to age, £675 per annum at 16, £844 at 18, £1,397 at 25 or over to £1,612.

Applications in own handwriting stating age, qualifications, experience, naming two referees and quoting EM/SA, should be sent to the Administrative Officer, The Laboratory, Citadel Hill, Plymouth PL1 2PB, by February 20, 1974. (763)

Northumbrian Water Authority

Manager—Fisheries

£4860–£6700

The person appointed will be directly responsible to the Assistant Director of Scientific Services.

Fisheries Officers (2)

£3846–£4737

Applications are invited from persons at present employed in the Water Industry, as defined in circular 5/73 of the Water Services Staff Commission.

Further details and application forms are obtainable from Secretary and Director of Administration, Northumbrian Water Authority, Monitor House, Coast Road, Wallsend, Northumberland NE28 7RD.
(Please state present post and employer.)

Closing date: Monday, 4th March, 1974.

(768)

Scientist

required for interesting Biomedical Automation Project in the New Charing Cross Hospital to take charge of the developments in cell processing techniques for automated cancer cell scanning machines.

Qualifications and Experience: 1st or 2nd Class Honours in a Science Degree. Some development or research experience, especially in the realms of biochemistry, cytochemistry, cell biology or specialised microscopy would be an advantage.

Salary: £1,704 to £3,375 p.a. plus London Weighting.

Applications to Consultant in Charge, Regional Cytology Centre, Group Path. Lab., St. Stephen's Hospital, Chelsea, SW10 9TH.

(725)

DEPARTMENT OF BIOLOGY

LAKEHEAD UNIVERSITY

Applications are invited from established researchers for the post of FULL PROFESSOR to fill a senior position in the department. Field in environmental biology desirable. The successful candidate will be expected to have experience in graduate student supervision and to contribute to the development of an M.Sc. programme. Duties will commence July 1, 1974, and salary negotiable. Present minimum Full Professor $20,130 (1973 Scale).

Applications and (inquiries) along with Curriculum Vitae and names of 3 referees should be sent to:

Mr. D. E. Ayre, Secretary,
Lakehead University, Thunder Bay, Ontario P7B 5E1, Canada.

(767)

HOECHST PHARMACEUTICAL RESEARCH

PHARMACEUTICAL RESEARCH TECHNICIANS

These appointments offer the chance to join a new and expanding organisation which is part of one of the world's largest chemical companies. Currently we are seeking to appoint qualified Technicians to undertake research, in the areas of drug metabolism, pharmacokinetics and diagnostic reagents, at our recently completed £2 million pharmaceutical research laboratories.

Applicants should possess ONC, HNC, MI.Biol, GRIC. or equivalent qualification. Our approach is a broad multi-disciplinary one and vacancies exist for persons with basic training in chemistry, pharmacology, clinical chemistry, pathology or immunology. Experience in one of the following research areas would be a distinct advantage—metabolite isolation and identification, plasma assays of drugs or hormones, distribution studies with radiolabelled drugs, biochemical pharmacology and radio immunoassay.

The posts are based at Milton Keynes, a new city in a pleasant rural location, convenient to London by both rail and road. Rented accommodation can normally be made available to staff moving to the area and removal assistance will be given.

Facilities for day release and further education are excellent and the company's policy is to encourage personal advancement. An excellent salary will be paid and first-class benefits include annual merit bonus, contributory pension and free membership of Private Patients Plan.

Please write, with full details of career to date, to:
Mr. S. Friedlander, Personnel Officer,
Hoechst Pharmaceutical Research Limited,
Walton Manor, Walton, Milton Keynes, Bucks MK7 7AJ.
Tel.: Pineham 5068.

(759)

Wellcome

Chemist/Biochemist

Biological Reagents

A chemist or biochemist, preferably with some experience of clinical chemistry, is required to assist with the production and development of an extensive range of Quality Control products and procedures in our Department of Biological Reagents. After a sufficient period of familiarisation the successful candidate will be expected to take control of a production team and will then be directly responsible to the Production Manager, although he will still be able, and expected, to undertake related research and development activities.

The post requires a good theoretical knowledge of the analytical methods used in current clinical laboratory practice and the person appointed would be expected to keep himself informed of future developments in the field as they arise. Active contact and collaboration with clinical chemists throughout the UK and abroad is an integral part of the job.

Applications, giving brief details of age, qualifications and experience should be sent to the

Personnel Manager,
The Wellcome Research Laboratories,
Langley Court,
Beckenham,
Kent BR3 3BS.

1971

(731)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
(UNIVERSITY OF LONDON)

Department of Zoology and Applied Entomology

LECTURER IN ENTOMOLOGY

Applications are invited for the post of Lecturer in Entomology, tenable from October 1, 1974. Candidates should hold an honours degree in Zoology, preferably with postgraduate experience in systematic entomology. An interest in evolutionary taxonomy or in the taxonomic application of biochemical or serological techniques would be an advantage.

Salary on the scale £1,929 to £4,548 per annum plus £162 London allowance and F.S.S.U. benefits.

Applications, which should be accompanied by a curriculum vitae and the names and addresses of two referees, or requests for further information should be addressed to Professor T. R. E. Southwood, Head of Department of Zoology, Imperial College, London S.W.7.

The closing date for applications is April 8.

(734)

CSIRO
AUSTRALIA

DIVISION OF APPLIED GEOMECHANICS

RESEARCH APPOINTMENTS

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

The Division of Applied Geomechanics undertakes a wide range of studies in rock mechanics and in soil mechanics. The research programme includes both basic and applied projects, the latter being orientated towards problems in both the mining engineering and civil engineering fields.

The work of the Division is organized into three main programmes:—

DEFECT STRUCTURE MECHANICS

This programme emphasises the basic role that discontinuities play in the mechanical behaviour of soil and rock masses. The work tends to be of a longer-term nature and the importance of the relevant scientific disciplines is recognized.

GEOTECHNOLOGY

The work in this programme relates directly to requests from industry for assistance, usually with time restraints imposed. The work tends to be of a shorter-term nature often involving appraisals of the current state-of-the-art for the relevant techniques.

INTEGRATED STUDIES

In this programme opportunities are taken to test novel methods of design or analysis by field measurement of the mechanical response of engineering structures and excavations. These measurements are related to predictions of response and the measurement of the relevant properties of the earthen materials. Conclusions are made regarding the validity of the design and analysis methods.

An expansion of the Division's work is now in progress and additional research staff of the highest calibre are to be appointed in the following areas:—

FIELD: SUPPORT OF UNDERGROUND OPENINGS

Reference Number: 920/167

An opportunity exists to develop and evaluate methods of supporting underground openings. These include the use of pillars, and techniques such as bolting and dowelling. The appointee will be responsible for relating research findings to the questions of safety and economy in developing underground openings for mining and civil applications.

FIELD: SLOPE STABILITY

Reference Number: 920/168

Opportunities exist to participate in research programmes aimed at developing rational design methods for open pit slopes, earth and rock fill dams and similar earthen structures. The appointee will plan and pursue programmes of laboratory and field testing of earthen materials, mathematical and physical model studies, field instrumentation and analysis of field behaviour with the aim of defining design procedures.

FIELD: ROCK FABRIC

Reference Number: 920/169

An opportunity exists for a structural geologist to take part in a programme of investigations into the influence of geometrical fabric features on the mechanical behaviour of rock masses. The appointee will develop concepts of quantitative fabric description related to models of mass behaviour and development of design principles.

FIELD: MEASUREMENT OF STRESS DEFORMATION BEHAVIOUR OF EARTHEN MATERIALS

Reference Number: 920/170

An opportunity exists for innovations—at basic and developmental levels—in the design of instruments and equipment for the measurement of all physical variables relevant to the definition (directly or indirectly) of the stress-deformation behaviour of earthen materials. The appointee will be expected to be familiar with modern techniques of instrumentation and to become familiar with the real physical characteristics of earthen masses.

FIELD: FRACTURE AND FRAGMENTATION STUDIES

Reference Number: 920/171

An opportunity exists to investigate the geomechanics aspects of the fracture and fragmentation of earthen materials, particularly rocks. The scope of this position includes basic studies on the role of stress path history and the application of findings to examinations of rock breaking techniques and equipment.

FIELD: GEODYNAMICS

Reference Number: 920/173

An opportunity exists for a geophysicist or engineer to develop methods of analysis of the mechanical response of soils and rocks subject to dynamic excitation such as seismic events, blasting, and rock bursts. The studies include theoretical and experimental aspects and apply to the stability of engineering structures and excavations. The work also includes the use of dynamic response patterns in the analysis of stress or strain conditions in earthen materials.

FIELD: GEOTECHNICAL PROCESSES

Reference Number: 920/174

An opportunity exists to study the ways in which geotechnical processes can be used to modify the mechanical response of soil and rock masses. These processes may include reinforcing, anchoring, cementation, chemical treatment, grouting, thermal and electrical techniques, boring and cutting, and the use of slurries.

QUALIFICATIONS

A Ph.D. degree or equivalent qualifications, together with demonstrable research ability.

SALARY AND TENURE

Appointments will be made within the salary ranges of:—

Research Scientist— $A9,380—$A11,827 p.a.
Senior Research Scientist—$A12,298—$A14,290 p.a.
Principal Research Scientist—$A14,882—$A17,077 p.a.

Outstanding applicants may be offered appointments within the salary range of:—

Senior Principal Research Scientist—$A18,077—$A20,005 p.a.

and in such cases the successful applicants may be required to accept positions of responsibility in the leadership of the Divisional research programmes, i.e. Defect Structure Mechanics, Geotechnology, and Integrated Studies.

Either an indefinite or a fixed term appointment may be negotiated. Indefinite appointments carry Australian Government Superannuation benefits.

APPLICATIONS

Applications stating full personal and professional details, the names of at least two professional referees, and **quoting the appropriate Reference Number** or enquiries seeking further information about these positions should reach:—

The Personnel Officer,
Australian Scientific Liaison Office,
64–78, Kingsway,
LONDON, WC2B 6BD.
by the **26th April, 1974.**

Applications in U.S.A. and Canada should be sent to:—
The Counsellor (Scientific),
Embassy of Australia,
1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.

(769)

Tropical Products Institute

ELECTRICAL ENGINEER

Culham, Berks ■ Ref SA/12/JD: Design/development of plant and equipment for processing fruit, vegetables and other renewable resources of developing countries. Responsibility for conducting trials of completed plant at Culham and commissioning trials overseas.
□ HNC/HND/degree in Electrical Engineering □ Experience of drawing office work and knowledge of electrical and/or electronic mechanical instrumentation an advantage □ Age under 30 □ Appointment as Higher Scientific Officer.

FOOD TECHNOLOGIST/ENGINEER

Culham, Berks ■ Ref SA/13/JD: Supervise project work in processing of non-cereal foods. Pilot plant studies at Culham followed by field trials overseas.
□ Good honours degree, or equivalent, in relevant subject □ 5-10 years' experience of food processing and process development □ Age under 32 □ Appointment as Senior Scientific Officer.

WOOD SCIENTIST

Culham, Berks ■ Ref SA/16/JD: Join multidisciplinary team studying problems of industrial utilisation of cellulose materials of tropical origin, with emphasis on small scale use of forest products and agricultural residues. Opportunity to develop own ideas. Responsibility for work of support staff.
□ Degree/HNC or equivalent in Wood Science, Chemistry or Timber Technology □ Age under 27 □ Appointment as Scientific Officer.

PULP AND PAPER TECHNOLOGIST

London ■ Ref SA/11/JD: Assess potential of fast-growing materials for paper pulp in developing countries. Chemical analysis; semi-chemical and mechanical pulping trials; pulp evaluation and consideration of economics involved.
□ HNC or equivalent in relevant subject □ Broad knowledge of papermaking an advantage □ Age under 30 □ Appointment as Higher Scientific Officer or Scientific Officer.

MICROBIOLOGICAL CHEMIST

London ■ Ref SA/14/JD: Develop study of chemical changes associated with microbial spoilage of food. Initial emphasis on bacterial spoilage of tropical freshwater fish. Opportunity to work overseas.
□ 1st/2nd honours degree in Chemistry, Biochemistry or Microbiology □ Experience in application of modern analytical techniques to biological problems an advantage □ Age under 27 □ Appointment as Scientific Officer.

BIOLOGIST/BIOCHEMIST

London ■ Ref SA/15/JD: Metabolic studies on tropical fruit and vegetables, related to storage, handling, transport and quality. Instrumental methods of analysis/investigation employed.
□ Degree or equivalent in Biochemistry, Biology or Chemistry □ Experience of modern instrumental techniques highly desirable □ Age under 27 □ Appointment as Scientific Officer.

VEGETABLE OIL TECHNOLOGIST

London ■ Ref SA/20/JD: Join section assisting developing countries to utilise their resources of fats, oilseeds and edible nuts. Applied research to improve these commodities and introduce new varieties. Considerable amount of field work overseas.
□ 1st/2nd honours degree in Chemistry or Biochemistry □ Experience of work with oilseeds an advantage □ Age under 32 □ Appointment as Senior Scientific Officer.

PESTICIDE ANALYST

London ■ Ref SA/21/JD: Estimate pesticide residues on crop and environmental samples obtained as a result of R & D programmes overseas. Assist training of chemists from developing countries in pesticide analysis. Possibility of short-term assignments overseas.
□ HNC/degree, or equivalent, in Chemistry □ Experience of analytical techniques, including gas chromatography an advantage □ Age under 27 □ Appointment as Scientific Officer.

SCIENTIFIC P.R.O.

London ■ Ref SA/19/JD: Arrange to show visitors around the Institute, plan and participate in exhibitions and prepare copy for publicity material. Assist in the organisation of seminars, conferences and open-days.
□ Degree, or equivalent, in scientific subject □ Ability to plan presentation of publicity material or an aptitude for composing press releases □ Age under 27 □ Appointment as Scientific Officer.

CROP STORAGE ENGINEER

Slough, Bucks ■ Ref SA/17/JD: R & D and advisory work on structures and systems for the handling and storage of cereal grains and other crops in developing countries. Responsibility for supervision of support staff. Assist in training of overseas students. Opportunity for short-term assignments overseas.
□ Good honours degree, or equivalent, in Agriculture or Civil Engineering □ 4 years' postgraduate experience □ Knowledge of agricultural crop production, storage systems and simple structural design work desirable □ Age under 32 □ Appointment as Senior Scientific Officer.

TRAINING OFFICER – FOOD STORAGE

Slough, Bucks ■ Ref SA/18/JD: Assist organisation of training programmes, on all aspects of storage for grain and similar crops, for students from developing countries.
□ Degree, or equivalent, in Natural Science or Agriculture □ Experience in organising course work an advantage □ Age under 30 □ Appointment as Higher Scientific Officer or Scientific Officer.

SALARIES: (London) SSO £2790-£3815; HSO £2251-£2842; SO £1493-£2352; (Culham/Slough) SSO £2615-£3640; HSO £2076-£2667; SO £1318-£2177.
Application forms (to be returned by 8th March 1974) from Miss C. A. Hall, Administrative Secretariat, Tropical Products Institute, 127 Clerkenwell Road, London EC1, please quote appropriate reference.

(740)

Roussel Laboratories Ltd. require

Biochemist

PHARMACEUTICAL RESEARCH

Roussel Laboratories Ltd., is engaged in the research, development and manufacture of a wide range of pharmaceutical and allied products, within the international Roussel-Uclaf group. We have an enviable record of rapid growth and are currently planning for a further phase of expansion.

We are now looking for a young Biochemist at our modern Research & Development laboratories in a pleasant part of Wiltshire.

He or she will join a small creative team developing new biochemical methods of testing potential drugs. The work also involves routine studies of the metabolism and distribution of new drugs using radiotracer techniques and automated equipment.

Applications are invited from graduates with some experience of Clinical Biochemistry or Biochemical Pharmacology, who would welcome the opportunity of proving their versatility and creativity.

Our conditions of service are excellent and a competitive salary will be offered, together with relocation assistance where appropriate.

Please write concisely, with details of qualifications and experience to:

Miss R. Curtis, Personnel Officer,
Roussel Laboratories Ltd., Kingfisher Drive,
Covingham, Swindon, Wiltshire.

(736)

ROUSSEL

BHP Australia

Melbourne Research Laboratories (Instrumental)

Analytical Chemist

BHP Melbourne Research Laboratories are the product research centre for the BHP Group and operate in the fields of metallurgy, structural engineering and chemistry. The Laboratories were established in 1969 near Monash University in Clayton, Victoria.

An experienced instrumental analyst is required to take responsibility for the provision of a high quality analytical service from an instrumental laboratory comprising a direct reading optical emission spectrograph, a sequential XRF spectrometer, a vacuum fusion mass spectrometer (gases in metals analysis). Methods development ability is also required.

Candidates are expected to be capable of deputising for the Group Leader, Materials Analysis.

General Conditions: Salary will be negotiated on the basis of qualifications and experience. General conditions of service include superannuation, additional medical benefits and an annual salary review. Free passage to Australia for appointee and family. Subsidised housing on arrival plus settling-in allowance.

Suitable candidates are invited to write to the undermentioned address for an application form. Applications will be treated in the strictest confidence.

Mr. A. S. Cornell, Administrative Manager – London,
The Broken Hill Proprietary Co. Ltd., Hanover House, 14 Hanover Square, London W1R 0ND.

(761)

TECHNICIEN SUPERIEUR BIOLOGISTE

Les laboratoires Pfizer recrutent pour leur Centre de Recherche à Amboise, France, Vallée de la Loire, un technicien supérieur biologiste, homme, âge minimum 40 ans, possédant une expérience d'au moins 10 ans du travail de routine de pathologie et une expérience de plusieurs années de la supervision des techniciens et de la gestion d'un service de pathologie. Le candidat sera responsable d'une équipe d'au moins 15 techniciens et devra pratiquer couramment le français et l'anglais. Un salaire élevé sera offert au candidat présentant les conditions requises.

Envoyer C. V. et prétentions à Dr H. Reinert, Directeur,
Centre de Recherche, Pfizer, B.P. 42, 37400 Amboise, France.

Pfizer

(743)

UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL

Research Assistant

required for microbiological studies in the aetiology of non-specific genital infections. The work is supported by a Medical Research Council grant. Salary either on Biochemist B scale or Whitley Council Technician scale depending on age, experience and qualifications. Experience with tissue culture an advantage but not essential.

Applications in writing, together with full curriculum vitae and the names of 2 referees, should be sent to the Secretary, University College Hospital Medical School, University Street, London, WC1E 6JJ, quoting reference PR/BACT. (721)

Coláiste na hOllscoile Gaillimh
University College, Galway
Department of Zoology
TECHNICIAN/SENIOR TECHNICIAN

Applications are invited for the above positions. Candidates for the post of Technician should possess the Intermediate Certificate of the City and Guilds or equivalent qualifications. The Advanced Certificate or equivalent qualifications will be required for the post of Senior Technician.

Salary: Technician—£1665 × £72—£1953
Senior Technician—£2034 × £78—£2346

Applicants should write to the Professor of Zoology for full particulars, including facilities available and duties applicable to the post.

Closing date—18th March 1974.

Department of Ophthalmology
TECHNICIAN

A vacancy exists in the Department of Ophthalmology for a Technician.

Minimum qualifications: Intermediate Certificate of Institute of Medical Laboratory Technology, Intermediate Certificate of City and Guilds of London, Intermediate Technological Certificate of Department of Education or equivalent.

Salary: £1665 × £72—£1953

Applications, stating age, experience, qualifications and the names of two referees, should be sent to Professor of Ophthalmology, Regional Hospital, Galway. Closing date: 18th March 1974. (728)

THE QUEEN'S UNIVERSITY OF BELFAST
LECTURESHIP IN MECHANICAL ENGINEERING

This post is tenable from October 1, 1974, or such later date as may be arranged. Candidates should have interests in the field of Materials Technology and a knowledge of and experience in the use of non-metallic materials would be an advantage. Salary scale is £1,929 to £4,548 with contributory pension rights under the F.S.S.U. Initial placing on the salary scale will depend on qualifications and experience.

Applications should be received by March 8, 1974. Further particulars may be obtained from: The Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. (Please quote Ref. 74/N.) (738)

UNIVERSITY OF NEWCASTLE UPON TYNE
CHAIR OF AGRICULTURAL BIOLOGY

The University is about to consider the appointment of a Professor of Agricultural Biology. The vacancy arises on the resignation of Professor Watkin Williams as Professor of Plant Science, and the change in title takes into account the University's decision to merge the existing Departments of Agricultural Zoology and Plant Science, in October 1975. The degree courses in Agricultural Zoology and Plant Science are to be maintained, and applications are invited from candidates sympathetic to this aim. It is recognised that no candidate's personal interests are likely to cover the whole field of the combined Departments. The Professor will be expected to take up his appointment as soon as possible. Salary in accordance with the Professorial scale (£5,625 by £96 to £5,721 by £195 to £6,501) with membership of F.S.S.U.

Further particulars may be obtained from the Registrar of the University, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU, with whom applications (15 copies), giving the names of not more than three referees, must be lodged not later than March 23, 1974. (Applicants from outside the British Isles may submit one copy only.) (750)

UNIVERSITY OF SURREY
DEPARTMENT OF BIOLOGICAL SCIENCES
TECHNICIAN GRADE 5
£2,007–£2,382

Technician Grade 5 required in the Human Biology section of the Department of Biological Sciences. The post involves assistance with class preparation and research, mainly in the field of immunology, with some microbiology and physiology.

Applicants should be suitably qualified and have some experience of immunological (serological) techniques. Experience in microbiological or physiological methods would be useful.

Application forms may be obtained from the Assistant Secretary (Personnel), University of Surrey, Guildford, Surrey GU2 5XH, or Tel. Guildford 71281, Ext. 452, and to whom they should be returned to by March 8, 1974. (752)

UNIVERSITY OF SHEFFIELD
THE MANCHESTER-SHEFFIELD SCHOOL OF PROBABILITY AND STATISTICS
APPOINTMENT OF TWO LECTURERS

Applications are invited for the above posts, tenable from October 1, 1974. Candidates may be specialists in any area of Probability and Statistics but some preference will be given to theoretical statisticians and applied probabilists. Salary in the range £1,929 to £4,548 with F.S.S.U. provision.

Further particulars from the Registrar and Secretary, The University, Sheffield S10 2TN, to whom applications should be sent by February 28, 1974. Please quote reference R36/G. (727)

LIVERPOOL POLYTECHNIC
SCHOOL OF PHARMACY
PRINCIPAL LECTURER IN PHARMACOLOGY

Applications are invited from graduates in Pharmacy or Pharmacology, who have a higher degree together with appropriate teaching and research experience, for appointment as Principal Lecturer in Pharmacology. The person appointed will have overall responsibility for the Pharmacology section of the School of Pharmacy, for the development of undergraduate work in Pharmacology and will be expected to promote postgraduate studies and research work. Excellent laboratory facilities are available and a new animal house is nearing completion. Salary: £3,581 to £4,499 per annum.

Details available from Staff Officer, Dept. 'N', Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool L3 9RH. (755)

Veterinary Appointments

The Company's research station for animal sciences undertakes a range of activity in the development of new products in the fields of animal health, hygiene and nutrition. There are major specific projects as well as general veterinary practice associated with the health of research animals on the station.

Consequent upon the re-organisation of objectives of the animal science research of the Company, vacancies have arisen for two Veterinary Surgeons.

Head of Product Evaluation

A senior Veterinary Surgeon is required to lead a team of scientists employed in the development of animal health, hygiene and nutritional products. The team will undertake the innovation of new products for the U.K. market and the work will involve extensive field trials. The team is also required to work closely with the research unit in the testing of new compounds and in carrying out field evaluation on products deriving from this work.

Veterinary Research Officer

A recently qualified veterinarian is required to join a team of research scientists involved in the screening and identification of new compounds active in the field of growth promotion, feed utilisation and parasitology. He will be responsible for a sector of research work in the growth promotion field and will also have general responsibility for the health of both research and farm animals,

The Thurgarton Research Station is located in rural surroundings between Nottingham and Southwell and has excellent field and laboratory facilities alongside the Boots farms which provide opportunity for extensive field testing. Later stages of research also involve trials throughout the U.K. and in overseas locations.

Terms and conditions of employment are attractive and include profit sharing and help with re-location. There is ample scope for career development in both the scientific and commercial sectors.

Interested applicants should apply to J. L. Muncey, Development & Training Manager, The Boots Company Limited, Head Office, Nottingham, with details of their career to date. Further information on the position involved can be provided on request.

Boots

The Boots Company Ltd.,
Nottingham NG2 3AA.

(730)

Research Associates are available for:—

Experimental and Theoretical High Energy Physicists

to join experimental and theoretical groups at Daresbury Laboratory. The experimental programme is based on the 5 GeV electron accelerator at Daresbury and on the various CERN facilities. The experimental and theoretical programmes are carried out in association with University physicists. Posts are normally available for a fixed term of three years.

The successful applicants will be appointed at salaries between £1,789 and £4,578. The level at which an Associate is appointed is dependent on age, ability and experience.

Applicants should possess a Ph.D in high energy physics or expect to obtain their Ph.D during 1974.

Superannuation is contributory under the Federated Superannuation System for Universities.

Closing date: 15 March 1974.

Please write or telephone Warrington 65000, Ext 467, for an application form quoting reference DL/502/T to: (784)

SCIENCE RESEARCH COUNCIL DARESBURY LABORATORY

Personnel Officer,
Science Research Council
Daresbury Laboratory,
Daresbury,
Warrington WA4 4AD.

CORPORATE LABORATORY—RUNCORN

ICI

The Corporate Laboratory has a vacancy for a

BIOCHEMIST

With wide range technical skills, and the originality and flexibility to make important contributions to a number of exploratory biological research projects. Applicants should be under 30 and hold a PhD in Bio-Chemistry.

The Laboratory is an interdisciplinary community where scientists have considerable freedom in carrying out exploratory research. Initiative and creativity are, therefore, the key requirements, and breadth of outlook is more important than specialised experience.

Runcorn, conveniently situated for Manchester, Liverpool and Chester, is within easy reach of the Lake District, North Wales and the Peak District, yet is only 2½ hours by train from London.

Please write for application form to:

Mr. J. A. Hurst,
Personnel Officer,
I.C.I. Limited, Corporate Laboratory,
P.O. Box 11,
The Heath, Runcorn, Cheshire WA7 4QE.

(737)

GRADUATE IN AGRICULTURE

Required by

AN FORAS TALUNTAIS

(The Agricultural Institute) in the Dairy Husbandry Department at its Moorepark Research Centre, Fermoy, Co. Cork. The appointee will be engaged in research on the development of intense dairy farming systems with particular emphasis on grassland production and utilization. Appointment up to and including Senior Research Officer level depending on qualifications and experience. Marriage and children's allowances. Non-contributory superannuation scheme and provision for widows and orphans pensions.

Essential: (1) Honours University Degree or equivalent in Agriculture. (2) Suitable research experience.

Application forms and further particulars from: The Personnel Department, (Ref.: R.D.H.), An Foras Taluntais Headquarters, 19 Sandymount Avenue, Dublin 4. Tel.: 693222.

Latest date for receipt of completed forms, Thursday, February 28, 1974. (756)

UNIVERSITY OF THE ORANGE FREE STATE

CSIR FLAVONOID RESEARCH UNIT

RESEARCH ASSISTANTSHIP

Applications are invited for the above vacancy in the Department of Organic Chemistry tenable over a period of up to 3 years. The work involves postgraduate research in all branches of flavonoid and tannin chemistry leading to M.Sc. or Ph.D. degrees, according to initial qualification. Preference will be given to candidates for the Ph.D. degree. The position should be assumed as soon as possible after January 1, 1974.

Minimum initial salary with M.Sc. qualification: R3,000. B.Sc.(Hons.): R2,500.

Applications giving details of academic record and curriculum vitae should be posted to Prof. D. G. Roux, Department of Chemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, South Africa. (746)

ROYAL POSTGRADUATE MEDICAL SCHOOL

BIOCHEMIST required to take technical charge of a biochemical laboratory in the Urological Unit which has a special interest in the renal dialysis and kidney transplantation. The laboratory is well equipped, automated, and there will be ample opportunities for original research. Salary according to qualifications and experience.

Applications to the Secretary, R.P.M.S., Hammersmith Hospital, DuCane Road, London W12 0HS, quoting Ref. No. 3/107/N. (715)

UNIVERSITY OF LEICESTER

DEPARTMENT OF ARCHAEOLOGY

TECHNICIAN IN TECHNICAL ARCHAEOLOGY

Salary Technician Grade IV: £1,848 to £2,163

Applications are invited for the above post from those preferably holding the Diploma in Conservation of the London University Institute of Archaeology or equivalent qualifications and experience in the entire range of archaeological conservation. The successful candidate will be in charge of the day-to-day running of the Department's extensive laboratories, workshop and dark-room facilities.

Applications (including the names of not more than two referees) to Professor J. V. S. Megaw, Department of Archaeology, University of Leicester, LE1 7RH, from whom further particulars may be obtained. Applications must be received by April 1, 1974.

(717)

INFORMATION OFFICER
(S.O. GRADE)

required to provide technical information and advice and to assist the Head of Research and Development Division. He or she must be able to write clear and concise reports in good English. A degree in a biological discipline is essential, preferably including botany or agricultural botany, with chemistry or biochemistry as subsidiary subjects.

Salary within the scale £1,824 to £2,796 per annum, according to age, qualifications and experience, plus London Allowance. Contributory pension scheme. Luncheon vouchers.

Application form from Personnel Officer, Potato Marketing Board, 50 Hans Crescent, Knightsbridge, London SW1X 0NB. (709)

BRITISH ANTARCTIC SURVEY

A GRADUATE METEOROLOGIST/PHYSICIST is required to develop research at isolated micrometeorological stations on glaciers in the Antarctic. The stations are taking part in an International Hydrological Decade study of the heat, ice and water balances of selected glaciers in differing environments in many parts of the world. In addition to research experience, candidates must be competent to maintain and repair radiation and electronic recording instruments. They must be physically fit and suited to an outdoor life involving sledging, skiing and flying.

The successful candidate will spend two years in the Antarctic before returning to the United Kingdom to prepare his results for publication. In appropriate cases research findings may be submitted for a higher degree.

If YOU are interested in seeing a largely unknown, remote and fascinating part of the world please write to:

The Establishments Officer,
British Antarctic Survey,
30 Gillingham Street,
London SW1V 1HY.
Tel.: 01-834 3687. (729)

UNIVERSITY COLLEGE OF WALES, BANGOR

DEPARTMENT OF PHYSICAL OCEANOGRAPHY

RESEARCH ASSISTANT

Applications are invited for the above post in a small research team investigating the circulation of the Irish Sea. The programme of research, which is sponsored by N.E.R.C., involves the development of radio located buoy systems and their application to the evaluation of numerical models of the circulation. The appointment will be in the first instance for two years commencing as soon as possible.

Applicants should have a good working knowledge of electronics, preferably, though not necessarily, with experience of R.F. transmission. Previous experience of work at sea is also considered an advantage.

Salary will be in the range: £1,700 to £2,000 per annum depending on qualifications.

Applications (two copies), giving full details of qualifications and experience together with the names and addresses of two referees should be sent not later than March 15, 1974, to the Secretary and Registrar, University College of North Wales, Bangor. (723)

UNIVERSITY OF WINDSOR

WINDSOR, ONTARIO, CANADA

DEPARTMENT OF PHYSICS

FACULTY APPOINTMENT IN RELATIVITY

It is hoped to make the appointment at the rank of Assistant Professor or Associate Professor, with duties to begin on July 1, 1974. Preference will be given to applicants with particular qualifications and interests in the theory of relativity and its applications. The successful candidate will be expected to engage in the teaching of undergraduate and postgraduate students, and to participate in the research activities of the Relativistic Group.

Further information may be obtained from Dr L. Krause, Head of the Department. (704)

Corrected Advertisement

UNIVERSITY OF GLASGOW

DEPARTMENT OF CHEMISTRY

Applications are invited from POSTDOCTORAL BIO-ORGANIC CHEMISTS for a research position under an M.R.C. project grant to Drs C. J. W. Brooks (Chemistry) and W. A. Harland (Pathology) for biochemical studies of human atherosclerosis. The research depends on modern micro-analytical techniques, including combined gas chromatography-mass spectrometry. Experience in lipid or sterol chemistry would be advantageous. The post is available immediately. Initial salary in the range £1,929 to £2,223 per annum for suitably qualified candidates.

Applications with curriculum vitae, citing two referees, should be sent to Dr C. J. W. Brooks, Department of Chemistry, University of Glasgow, Glasgow G12 8QQ. In reply please quote Ref. No. 3408. (684)

QUEEN MARY COLLEGE
University of London

Research Posts for Studies of Electronic Excitations in Polymers

Physicists or chemists with experience in solid-state physics or of relevant chemical preparation methods are invited to write for details of posts for postdoctoral associates and experimental officers for research in either:

Spectroscopic studies of the characters of electronic excitations in chain-extended conjugated polymers, and of their migration behaviour.

Studies of submillimetre and millimetre-wave absorption processes in dielectric polymers with crystalline and chain-extended morphologies.

Enquiries (including brief outline of experience and interests) to Professor D. H. Martin (N), Queen Mary College, Mile End Road, London, E1 4NS.

(712)

British Museum (Natural History)

Botany [Computer Science]

■ Head of British Flowering Plant Section ■ Organise section's curatorial and advisory services ■ Research into and advise on computer methods for collection of systematic data and identification of plants.

□ 1st/2nd hons degree in Botany, computer science or equivalent □ 4 years' postgraduate experience □ Post suitable for botanist with computing experience or computer scientist with botanical background □ Age under 32 □ Appointment as Senior Scientific Officer (£2790–£3815) □ Ref: SB/17/DK.

Zoologist

■ In Crustacea Section ■ Undertake taxonomic research on Copepoda ■ Curatorial and advisory work on other entomostracan groups.

□ 1st/2nd hons degree, or equivalent, in Zoology □ Keen interest in taxonomic Zoology □ Age under 32 □ Appointment as Senior Scientific Officer (£2790–£3815), Higher Scientific Officer (£2251–£2842) or Scientific Officer (£1493–£2352) according to age, qualifications and experience □ Ref: SB/18/DK.

□ Application forms (for return by 18 March 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Science group
CIVIL SERVICE

(771)

WYE RIVER AUTHORITY

ASSISTANT FISHERIES OFFICER

Applications for the above post are invited from candidates with a degree in biology or zoology. In addition to duties of a biological nature, the successful candidate will be required to familiarise himself with, and undertake, administrative duties in the Fisheries Department. Experience with a River Authority, including working with Water Bailiffs, would be an advantage, but this is essentially a post for an officer who wishes to make a career in Fisheries Management. The salary will be on A.P. Grades 3/4 (£1,926-£2,535 per annum), starting point according to age and experience. An essential user car allowance will be paid. Applications, stating age, qualifications and experience, should be sent to the undersigned on or before March 7.

J. A. WESTON, Clerk of the Authority.

4 St. John Street, HEREFORD.

(710)

CSIRO

AUSTRALIA

DIVISION OF LAND RESOURCES MANAGEMENT

RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD:

SOIL BIOLOGY/NUTRIENT CYCLING

GENERAL: The Division, with headquarters in Perth and field stations at Deniliquin, N.S.W., Alice Springs, N.T., and Canberra, A.C.T., is concerned with the effects of land use on the productivity and conservation of land resources. A vacancy exists at the Alice Springs Field Centre for a Research Scientist in the field of nutrient cycling in arid ecosystems.

The Alice Springs Field Centre has an interdisciplinary research team of 4 professional scientists including plant and animal ecologists, a plant physiologist, and a hydrologist. It is also closely associated with various Branches of the Department of the Northern Territory which employ over 20 professional scientists.

Alice Springs is a modern town with a population of over 13,000 and is situated in the Macdonnell Ranges in Central Australia. It has good communications and secondary schooling is available. Housing will be arranged for a married appointee and hostel accommodation is available for a single appointee.

DUTIES: To study nutrient cycling and its role in maintaining soil fertility and ecosystem stability in arid Australia. This will be a vital addition to the existing interdisciplinary study of ecosystem functioning in the Alice Springs district. The opportunity also exists for developing cooperative projects with scientists engaged in similar studies at the Riverina Laboratory, Deniliquin, and at Headquarters in Perth.

QUALIFICATIONS: A Ph.D. degree, or equivalent qualifications, together with demonstrable research ability.

SALARY: Appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: $A9,380–$A14,290 p.a. In addition, a District Allowance of $A500 p.a. (married) or $A270 p.a. (single) is payable.

TENURE: The position is available for an indefinite period and Australian Government Superannuation benefits are available.

Applications, stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number: 284/15 should reach:—

The Personnel Officer,
Australian Scientific Liaison Office,
64–78, Kingsway, LONDON, WC2B 6BD by the **22nd March, 1974.**

Applications in U.S.A. and Canada should be sent to:—
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.

(770)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF PURE AND APPLIED CHEMISTRY

Lectureship in Chemical Technology

Applications are invited for a Lectureship in the Department of Pure and Applied Chemistry. Applicants should have experience in surface chemistry or colloid chemistry and an interest in the application of these subjects to industry and/or biology.

The appointment will be on the salary scale £1,929–£4,548 with FSSU benefits. The initial salary will depend on qualifications and experience.

Application forms and further particulars (quoting 10/74) may be obtained from the Registrar, University of Strathclyde, George Street, Glasgow G1 1XW, with whom applications should be lodged by 16th March, 1974.

(753)

UNIVERSITY OF ST. ANDREWS

DEPARTMENT OF PHYSIOLOGY

Applications are invited for a post of RESEARCH ASSISTANT to work on R.N.A. metabolism as part of an M.R.C. project award to study the effects of the cell nucleus on sodium pump production in cultured cells. The appointment will be for three years from October 1, 1974, at a salary of £1,000 per annum (by £50 to £1,100). The successful candidate will be able to register for a Ph.D. degree.

Applications with the names of two referees should be sent by March 31, 1974, to Professor J. F. Lamb, Department of Physiology, The University, St. Andrews, Fife, KY16 9TS, from whom further details are available. (757)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)

DEPARTMENT OF PHYSICS AS APPLIED TO MEDICINE

A RESEARCH ASSISTANT is required for a period of one year to work with a group investigating solid state (Xeroradiography and Ionography) X-ray ionizing methods. Salary up to £2,220 per annum.

Applications stating age, qualifications and experience, giving the names and addresses of two referees, to Professor J. F. Tait (N), Department of Physics as Applied to Medicine, The Middlesex Hospital Medical School, Cleveland Street, London W1P 6DB, by March 9, 1974. (714)

UNIVERSITY OF DURHAM

DEPARTMENT OF PSYCHOLOGY

Applications are invited from candidates with qualifications in the Biological Sciences or Comparative Psychology for the post of LECTURER IN PSYCHOLOGY from October 1, 1974.

The salary will be on the scale £1,929 to £4,548 per annum with F.S.S.U. benefits.

Applications (3 copies) including the names and addresses of three referees should be sent by March 11, 1974, to the Registrar and Secretary, Science Laboratories, South Road, Durham, from whom further particulars may be obtained. (718)

ROYAL POSTGRADUATE MEDICAL SCHOOL

TECHNICIAN/SENIOR TECHNICIAN required to take part in a study on the isolation of soluble mediators of delayed hypersensitivity reactions. Work involves in vitro cellular assays and a wide range of immunochemical techniques. Qualifications: B.Sc./H.N.C. or equivalent. Salary according to qualifications and experience.

Applications to the Secretary, R.P.M.S., Hammersmith Hospital, DuCane Road, London W12 0HS, quoting Ref. No. 21/218/N. (713)

UNIVERSITY OF READING

DEPARTMENT OF AGRICULTURAL BOTANY

RESEARCH DEMONSTRATORS

There are vacancies for graduates and for this year with good honours degrees in Botany, Agricultural Botany or a related subject, to pursue research in plant physiology, applied pathology, leading to a higher degree. Up to 8 hours demonstrating per week is required. The salary scale is £1,047 by £51 to £1,149 per annum and appointments will normally be for three years from October 1, 1974.

Further particulars are available from Professor Watkin Williams, Department of Agricultural Botany, Plant Science Laboratories, University of Reading, Whiteknights, Reading RG6 2AH. (Ref. T 17.)

(766)

ST. THOMAS'S HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)
LONDON SE1 7EH

GRADUATE RESEARCH ASSISTANT required to work in the Department of Anatomy assisting an investigation into the development of the blood supply to the human brain. The appointment is tenable for two years at an initial salary of £1,266 plus £126 London Weighting.

Applications in writing, with curriculum vitae and the names of two referees, should be sent to the Secretary. (773)

CELL BIOLOGIST or RADIOBIOLOGIST (post-doctoral preferred) for research on mutagenic and lethal effects of ionising radiations of differing quality on mammalian cells in culture. The post is situated at AERE Harwell and the successful candidate will be required to organise the cell culture laboratory and to plan and carry out experiments under the joint supervision of the Gray Laboratory of the Cancer Research Campaign, Northwood, and of the Environmental and Medical Sciences Division at Harwell.

The post is for a period of 3 years in the first instance, and the salary will be within the range of £1,500 to £3,000.

Applications, with curriculum vitae and the names of two referees, should be sent to the Director, Gray Laboratory, Mount Vernon Hospital, Northwood, Middlesex HA6 2RN, from whom additional information may be obtained. (708)

RECKITT & COLMAN

PHARMACEUTICAL DIVISION

An experienced Technician

is required for the Neuropharmacology Section. Applicants should possess H N C (Chemistry/Biology) or an equivalent qualification and preference will be given to persons with experience in relevant analytical techniques (such as G.L.C. and fluorimetry).

The successful candidate will be expected to contribute to the efforts of a team currently engaged in fundamental investigations concerning the biochemical basis of certain mental diseases.

Reckitt and Colman operates internationally and its pharmaceutical interests are based at Hull where first-class facilities are available. Over the next few years major investment is planned in an expansion of the Company's pharmaceutical research activities.

Salary is negotiable according to experience and qualifications. Fringe benefits include annual bonus and relocation assistance.

Will applicants please write giving details of qualifications, education and career to date to: C. E. Rhodes, Personnel Dept., Reckitt & Colman, Dansom Lane, Hull HU8 7DS. (748)

DEPARTMENT OF GENETICS

UNIVERSITY OF COLOGNE

A post is offered to a SENIOR RESEARCH FELLOW supported by the Sonderforschungsbereich 74 "Molecular Biology of the Cell". We are seeking a scientist, who works with **Eucaryotic cells or their subcellular systems** and who has sufficient experience to establish and coordinate an independent research group. Applicants should be interested in cooperating with other groups of the SFB. Their work includes problems of molecular biology of bacteria, research with animal tumour viruses and cellular biology of immune systems.

The salary corresponds to that of an associate professor. The position is initially for three years with possible extension to five years. Suitable laboratory space and an independent budget for personnel and operating expenses are provided.

Applications should be sent before May 31, 1974, to the speaker of the SFB 74, Prof Dr P. Starlinger, Institut für Genetik der Universität zu Köln, **5 Köln 41**, Weyertal 121, Germany. (745)

ROYAL POSTGRADUATE MEDICAL SCHOOL

SCIENTIFIC OFFICER (Graduate Biochemist) required to join research group investigating cellular and metabolic damage associated with tissue anoxia. The study is a joint project between the Cardiovascular Research Unit at the School and the Department of Biochemistry at Imperial College. Experience with isolated organ perfusion and spectrophotometric enzyme assays would be advantageous. Salary in range £1,812 to £2,448 per annum according to qualifications and experience.

Applications to the Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS, quoting reference 2/494/N. (764)

LECTURER IN BOTANY

Applications are invited for the above post to assist in teaching courses for B.Sc. in Biology, and possibly contributing to M.Sc. teaching. Candidates qualified in plant ecology, higher plant physiology or plant systematics will be given particular consideration, but those with other interests may apply. Initial salary on scale will not exceed £2,388 plus London allowance of £162 per annum.

Further details and forms of application should be obtained from the Secretary, Birkbeck College, Malet Street, WC1E 7HX. Closing date: March 18, 1974. (779)

PROFESSOR OF NEUROPHYSIOLOGY REquires EXPERIENCED TECHNICIAN to assist in servicing a practical classroom and in research. Grade 4 technician post during initial year pending retirement of existing Grade 5 holder of post.—Handwritten applications stating age, experience, qualifications and names of two referees, to Administrator, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT. (772)

UNIVERSITY OF LEEDS, DEPARTMENT OF Radiotherapy. Research Technician Grade 3 (£1,650 to £1,920) required to assist in experimental studies of the effect of radiation on biological materials at Leeds General Infirmary and Cookridge Hospital. Applicants should preferably have experience of mammalian cell and tissue culture techniques and an interest in cytogenetics. Applications, stating age, qualifications, experience and present salary together with the names and addresses of two referees, should be sent to: Dr. A. W. Lees, University Department of Radiotherapy, Cookridge Hospital, Leeds LS16 6QB, from whom further details can be obtained. (777)

HARWELL

INSTRUMENTATION AND CONTROL SYSTEMS

The Electronics and Applied Physics Division at Harwell has a vital role as a centre for advanced electronic knowledge, and as a source of specialised equipment, for the UK Atomic Energy Authority nuclear programme and for Harwell's research and development in the environmental, marine and medical fields. Our work ranges from initiating applied research aimed at anticipating future needs, to tackling immediate problems in close collaboration with workers in other disciplines. Our technology covers a broad range including measurement system design, transducers, small electrical energy sources, semiconductor technology, and signal processing and communication in both analogue and digital forms, often requiring knowledge of both hardware and software.

Within this range we are seeking additional research staff to be involved in:—

Systems application including development of digital hardware and associated software.

Advanced electronic circuit design and development, particularly for signal processing in instrumentation systems.

Applicants should have a clear interest in some of the above areas and should possess a good Honours Degree in Electrical/Electronic Engineering or in Physics; preference will be given to those with postgraduate experience in an appropriate area.

Appointments will either be Fellowships for three years or as permanent appointments. The stipend or salary will depend upon ability and experience and will be in the range of £1,600 to £3,500 per annum.

For application forms please write to Appointments Section 'A', A.E.R.E., Harwell or phone Abingdon 4141, Ext. 2058.

(735)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF CANCER STUDIES

RESEARCH ASSOCIATE

Applications are invited for a Research Associate to work on a study of virus expression in somatic cell hybrids. This study is part of a project examining tumour virus persistence and function, and is supported by the Cancer Research Campaign. Candidates should have a good degree in a biological science with experience in tissue culture and/or genetics. Salary will be on the scale £1,569 to £2,223.

Applications with curriculum vitae and names of two referees to Professor D. G. Harnden, Department of Cancer Studies, Medical School, Birmingham B15 2TJ, by March 11, 1974. (530)

M.R.C. CLINICAL RESEARCH CENTRE

(NORTHWICK PARK HOSPITAL)

WATFORD ROAD, HARROW, MIDDLESEX
HA1 3UJ

Division of Immunology require two post-doctoral or medical scientific workers.

Post A: to extend work on control mechanisms in immunology, taking as a starting point observations on immunological unresponsiveness in mice and the transient nature of the primary response.

Post B: to initiate basic studies on the aetiology of immunodeficiency disease in a setting in which clinical and laboratory aspects can be combined.

The appointment is for 3-5 years and the salary is dependent upon age and experience.

Further details and application forms may be obtained from Mrs. J. Tucker-Bull. Please quote ref. 118/1A/B and state position of interest. (542)

UNIVERSITY OF GLASGOW

DEPARTMENTS OF BOTANY AND CHEMISTRY

Applications are invited from post-doctoral bio-organic chemists for a research position under an M.R.C. project grant to Drs. C. J. W. Brooks (Chemistry) and W. A. Harland (Pathology) for biochemical studies of human atherosclerosis. The research depends on modern micro-analytical techniques, including combined gas chromatography-mass spectrometry. Experience in lipid or sterol chemistry would be advantageous. The post is available immediately. Initial salary in the range £1,929 to £2,223 per annum for suitably qualified candidates.

Applications with curriculum vitae, citing two referees, should be sent to Dr. C. J. W. Brooks, Department of Chemistry, University of Glasgow, Glasgow G12 8QQ. In reply please quote Ref. No. 3408 M. (684)

CHELSEA COLLEGE

UNIVERSITY OF LONDON

LECTURER IN APPLIED HYDROBIOLOGY

Applications are invited from environmental biologists or chemists with a mathematical background and having interests in water pollution, effluent treatment or related fields. The Lecturer will assist in the organisation and teaching of quantitative aspects of the M.Sc. course in Applied Hydrobiology and appropriate undergraduate courses and will also be expected to undertake research. Salary scale: £1,929 to £4,548 per annum plus £162 London Allowance.

Further particulars and application forms, to be returned by March 15, 1974, from the Personnel Officer (N), Chelsea College, Manresa Road, London SW3 6LX. (741)

Animal Nutritionist

Applications are invited for a permanent post in the Agricultural and Food Chemistry Research Division of the Northern Ireland Department of Agriculture.

The successful applicant will join a team engaged in research in the various aspects of ruminant nutrition. He may also be required to undertake teaching duties in Agricultural Chemistry in the Faculty of Agriculture and Food Science, Queen's University, Belfast.

Appointment may be at Senior Scientific Officer, Higher Scientific Officer or Scientific Officer level.

SSO Over 25 and under 32 years of age with a first or second class Honours Degree in Chemistry, Biochemistry or Agricultural Chemistry and at least 4 years postgraduate experience in research and/or teaching, preferably in relation to animal nutrition.

HSO Under 30 years of age with an Honours Degree as above and at least 2 years postgraduate experience.

SO Under 27 years of age with an Honours Degree as above.

SALARY SCALES SSO £2,615–£3,640
HSO £2,076–£2,667
SO £1,318–£2,177

Entry point to the relevant salary scale will be related to qualifications and experience.

Please write or telephone for an application form, quoting Ref. SB26/74/N to: Civil Service Commission Clarendon House, Adelaide Street, Belfast BT2 8ND (telephone 0232–44300, extension 26). Completed forms should be returned to arrive not later than 5th March, 1974.

(739)

NORTHERN IRELAND CIVIL SERVICE

E.E.C. RESEARCH CENTRE – ITALY

CHEMIST

(MASS SPECTROMETRY)

The Chemistry Division of the EEC Research Centre at Ispra (near Lake Maggiore in Italy) requires a chemistry graduate, under 30, with a sound experience of mass spectrometry, preferably gained after a post-graduate course in this field. The initial contract will be for two years, probably renewable for a further year.

The work is concerned with the current Ispra programmes on fissile materials and problems relating to the disposal of waste.

Application forms and further details of the terms and conditions of employment, which compare well with national scientific institutions, may be obtained from the Commission of the European Communities – Mr. A. Mattevi, Co-ordination Unit, GD EX/CCR/BERL 5/54B, Directorate for Personnel, 200 Rue de la Loi, Brussels, Belgium.

Completed application forms should be received at the above address before 18th March, 1974.

(760)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

COURTAULD INSTITUTE OF BIOCHEMISTRY

A YOUNG BIOCHEMIST is required to work as a Research Assistant in a team studying the synthesis and metabolism of serum lipoproteins in relation to heart disease. The post carries a salary of up to £1,371 per annum. The appointee may be registered for a higher degree.

Students who expect to obtain a good class degree this summer are invited to apply, submitting a curriculum vitae and the names and addresses of two referees to: Dr. G. L. Mills, Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 5PR. (765)

UNIVERSITY OF CAMBRIDGE

The Appointments Committee of the Faculty of Biology 'B' intend to appoint a UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF BIOCHEMISTRY and applications are invited for this office.

The initial appointment will be for three years from a date as soon as possible after June 1, 1974. The pensionable stipend for a University Demonstrator is on a scale of £2,058 to £2,718 a year with initial placing above the minimum where appropriate. The maximum tenure of a University Demonstratorship is five years. A grant is made towards removal expenses.

Candidates should send twelve copies of their application, together with the names of not more than three referees, to Mr G. R. Anderson, General Board Office, The Old Schools, Cambridge, CB2 1TT, to arrive not later than April 5, 1974. (749)

UNIVERSITY OF KEELE

POSTDOCTORAL RESEARCH ASSOCIATE

Postdoctoral Research Associate required in Department of Physics for S.R.C. sponsored research programme concerned with magneto-optic studies of defects and rare earth ions in oxide crystals. Previous experience in magnetic resonance and optical spectroscopy advantageous. Appointment from October 1, 1974, or earlier by arrangement. Salary £1,929 per annum.

Further particulars and application forms from the Registrar, The University, Keele, Staffs., ST5 5BG, to whom completed applications should be returned by March 22, 1974. (751)

UNIVERSITY COLLEGE OF WALES

ABERYSTWYTH

DEPARTMENT OF AGRICULTURE

A.R.C. RESEARCH ASSISTANT required from July 1974 to work on winter cauliflower production.

Details may be obtained from the Registrar to whom forms should be returned by March 4, 1974. (720)

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOGY

A GRADUATE ELECTRON MICROSCOPIST is required to become part of a cancer research team. Technical assistance available.

Applications to Dr R. Borland, Department of Pathology, Tennis Court Road, Cambridge. Telephone 58251. (705)

CANCER RESEARCH CAMPAIGN

TECHNICIAN required for research on effects of drugs and radiation on cells in culture. 'A' levels advantage. Minimum salary £984 per annum aged 18, rising according to age and qualifications.

Apply Executive Officer, CRC Gray Laboratory, Mount Vernon Hospital, Northwood. (707)

UNIVERSITY OF TÜBINGEN

WEST GERMANY

There is a vacancy for a POST-DOCTORAL RESEARCH ASSISTANT in the Institute of Toxicology. The successful applicant will work in the field of neurobiology. Experience in immunology and/or histochemistry advantageous.

The appointment will be made for one year with a possibility of extension up to three years. Salary in range of $12,000 year.

For further particulars and application please write to: Dr. J. Schultz, Institute of Toxicology, University of Tübingen, Wilhelmstr. 56, West Germany. (786)

Applications are invited for a faculty position of CYTOGENETICIST with interest in culturing human gonadal cells. The person to be recruited would have a major commitment in education and research and about one-third of his/her time would be devoted to a service Cytogenetics laboratory.

Further particulars may be obtained from Dr L. A. Branda, Director Program in Reproductive Biology, McMaster Health Sciences Centre, Hamilton, Ontario, Canada L8S 4J9. (726)

POSTDOCTORAL RESEARCH ASSOCIATE to study the molecular mechanism of hormonal activation of adenyl cyclase using both membrane and whole cell systems. Experience in molecular pharmacology, membrane biology or cellular physiology required.—Applications with full curriculum vitae and names of two referees to: Dr R. M. Epand, Chemistry Department, University of Guelph, Guelph, Ontario, Canada. (747)

ANALYTICAL BIOCHEMISTS

These positions offer the opportunity to join a new and expanding organisation which is part of one of the world's largest chemical companies. Currently, we are seeking Analytical Biochemists to work at our newly completed, £2 million research laboratories at Milton Keynes.

Applicants should have experience of techniques used to estimate drugs or metabolites at very low levels in biological fluids and tissues. An interest in pharmacokinetics would be a distinct advantage.

An excellent salary will be paid and first class benefits include annual merit bonus, contributory pension scheme and free membership of Private Patients Plan.

Please write, with full details of career to date, to:

Mr S. Friedlander, Personnel Officer,
Hoechst Pharmaceutical Research Limited,
Walton Manor, Walton, Milton Keynes, Bucks MK7 7AJ.
Tel: Pineham 5068.

(758)

HOECHST

Kings
COLLEGE HOSPITAL GROUP

Cytogenetics
Department of Haematology

GRADUATE SCIENTIST

to work in the Genetic Laboratory of this busy teaching hospital. The post offers experience in the application of a wide variety of modern techniques to the diagnosis and management of inherited and malignant disorders. Experience in tissue culture or cytogenetics an advantage.

Application forms available from: Personnel Officer, King's College Hospital, Denmark Hill, London SE5 9RS. Tel.: 274 6222 Ext. 2724/8.

(722)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF CANCER STUDIES
RESEARCH FELLOW

Applications are invited from biochemists with an interest in tumour virology to join a group working on the detection of virus nucleic acids. The fellowship is supported by the Cancer Research Campaign. Candidates should be post-doctoral and have experience in nucleic acid chemistry. Salary for a Fellow is on the scale £1,929 to £2,223 but a higher point on the Lecturer scale would be considered for a well qualified candidate.

Applications with curriculum vitae and names of two referees to Professor D. G. Harnden, Department of Cancer Studies, Medical School, Birmingham B15 2TJ, by March 11, 1974. (529)

UNIVERSITY OF MANCHESTER

Applications invited from graduates in metallurgy, geology or physics to work on metallographic aspects of meteorite material using conventional metallographic methods in conjunction with a SEM 2 electron probe microanalyser. Appointment for one year in the first instance, renewable for up to three years. Salary range per annum: £1,929 to £2,388.

Applications (with curriculum vitae and names of two referees) to Dr. H. J. Axon, Department of Metallurgy, The University, Manchester M13 9PL. (699)

MONASH UNIVERSITY
MELBOURNE, AUSTRALIA
DEPARTMENT OF PHYSIOLOGY
(including Pharmacology)
LECTURER, SENIOR TEACHING FELLOW OR TEACHING FELLOW

The Department of Physiology conducts courses in Physiology and Pharmacology for B.Sc., B.Sc.(Hons.) and M.B., B.S. degrees and accepts graduate students for M.Sc. and Ph.D. degrees. Duties will relate especially to Science undergraduate teaching. Research interests include: brain ultrastructure, neurophysiology, biophysics and pharmacology of central and peripheral synapses, muscle biophysics, circulatory physiology and pharmacology, renal and endocrine physiology. Applicants for Senior Teaching Fellowship or Lecturer should preferably have a Ph.D. or medical qualification.

Salary scale: Lecturer, $A8,698 to $A11,982 per annum. Senior Teaching Fellow, $A7,270 to $A8,698 per annum, with superannuation based on an endowment assurance scheme, the employee and employer contributing 5 per cent and 10 per cent respectively. Teaching Fellow, $A5,740 to $A7,015 per annum.

Benefits: Travelling expenses for appointee and family; removal allowance; repatriation after three years' appointment if desired; temporary housing for an initial period. Study leave entitlement (for Lecturers only) accumulates at the rate of one month's leave for each six months' service up to six years with provision for financial assistance.

Further general information and details of application procedure are available from the Academic Registrar, Monash University, Wellington Road, Clayton 3168, Victoria, Australia, or the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF. Enquiries about the Department to the Chairman, Professor R. Porter, in the University.

Closing date: **February 28, 1974,** but will be accepted for a further 14 days.

The University reserves the right to make no appointment or to appoint by invitation. (744)

VERULAM GROUP HOSPITAL MANAGEMENT COMMITTEE
DEPARTMENTAL ADMINISTRATOR AND RECORDS OFFICER

required for the Kennedy Galton Centre, which provides clinical genetics services for the North West Metropolitan Region. This is a new post at the Centre, which is situated within the grounds of Harperbury Hospital.

Duties will include the organisation of supplies and stores, finance, personnel matters, patients' appointments, library and literature services and the reorganisation and development of the recording and retrieval system for patient data.

The appointment is on the General Administrative Grade, salary scale £1,686 to £2,187 per annum plus £126 London Weighting.

Application forms are available from the Group Secretary, Harperbury Hospital, Harper Lane, Shenley, Radlett, Herts WD7 9HQ. Closing date for return of applications: March 15, 1974. (733)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF PHYSICS
EXPERIMENTAL OFFICER
Superconducting AC Generators

Applications are invited for the 3-year appointment on a project supported by S.R.C. to study the thermal behaviour of liquid helium and evaluate refrigeration cycles under high speed rotation. Duties include the design, development and operation of cryogenic rigs rotating at 3,000 r.p.m. with diameters up to 1 metre.

Applicants should have a good degree in science or engineering or equivalent qualification.

Mechanical design experience with rotating machines would be an advantage, but previous cryogenic experience is not essential.

Salary scale £1,929 to £3,048 per annum plus F.S.S.U. benefits.

Applications in writing giving details of age, qualifications and experience and the names of two referees, should be sent to the Deputy Secretary's Section, The University, Southampton SO9 5NH, as soon as possible. Please quote reference number: N 192/R. (781)

FELLOWSHIPS AND STUDENTSHIPS

WELSH NATIONAL SCHOOL OF MEDICINE AND DEPARTMENT OF CHEMISTRY UNIVERSITY COLLEGE, CARDIFF
RESEARCH STUDENTSHIP

An opportunity exists for a graduate in physics, chemistry or biology to work for a higher degree. The project, which is sponsored by the Medical Research Council, is concerned with the development of polymeric materials capable of remaining on the tooth surface for at least six months.

A wide range of physical techniques is available in the Dental School and the Chemistry Department for the project. Students graduating in June 1974 may apply. This appointment will be for three years, salary £1,150 to £1,300.

Further particulars available from the Registrar, The Welsh National School of Medicine, Heath Park, Cardiff. (780)

AGRICULTURAL RESEARCH COUNCIL
FOOD RESEARCH INSTITUTE

Applications are invited for the award of an A.R.C. RESEARCH STUDENTSHIP tenable from October 1, 1974, at the Food Research Institute, Norwich, with a view to reading for a Ph.D. degree of the University of East Anglia.

The field of study will be in **either**

(a) The Microbiology Division working on one of two subjects:

- (i) the isolation, taxonomy and function of new groups of bacteria from the avian intestinal tract; or
- (ii) the structure and biochemical activities of the insoluble fractions of anaerobic bacteria.

OR

(b) The Chemistry Division working on the metal-catalysed oxidation of methyl esters of fatty acids.

The award comprises a tax-free maintenance allowance of up to a basic maximum of £695 (dependent upon marital status and accommodation occupied) plus other allowances for travelling, approved post-graduate experience, etc.

Candidates, who should normally be under 27 years of age, must have an appropriate degree with first or upper second honours.

Further details and application forms returnable by March 31, 1974, from the Secretary, Food Research Institute, Colney Lane, Norwich NOR 70F.

(762)

UNIVERSITY OF EXETER
DEPARTMENT OF ENGINEERING SCIENCE
RESEARCH IN BIO-ENGINEERING

A research studentship is available in the Department of Engineering Science, University of Exeter. The value of the studentship is £1,063 per annum and is available for a period of three years in the first instance.

The work will be concerned with an examination of the properties of bone, bone cement and implants under various loading conditions to determine the optimum properties of bone cements and the optimum method of transmitting loads through an implant into bone.

Applications are invited from science and engineering graduates with first or upper second class honours degrees.

The applications, giving details of the candidates' qualifications and experience, and the names of two referees, should be addressed to:

The Secretary of the University,
The University of Exeter,
Northcote House,
The Queen's Drive,
Exeter EX4 4QJ. (724)

ROYAL SOCIETY OF NEW ZEALAND

THE CAPTAIN JAMES COOK FELLOWSHIP

Applications are invited by the Royal Society of New Zealand for a Captain James Cook Fellowship, established by the New Zealand Government for research in New Zealand or the South West Pacific in the following subjects or any relevant field: anthropology, biology, geography, geology, geophysics, history, medicine, oceanography. The appointment, which will be made in September 1974 and should be taken up within six months thereafter, is for two years, but may be extended for a third year, and must be held at a university or research institution in New Zealand. The salary will be the maximum for an Associate Professor (at present $NZ12,300) together with travel expenses for the Fellow and his dependant family, baggage allowance, equipment grant and travel allowance to do the research proposed.

Applications, which must be received by the Royal Society of New Zealand by May 31, 1974, should be made on forms obtainable, with further details of the appointment, from the Executive Officer of the Royal Society of New Zealand or from either of the following:

The Executive Secretary,
The Royal Society,
6 Carlton House Terrace,
London SW1Y 5AG.

The Scientific Office,
New Zealand High Commission,
New Zealand House,
Haymarket,
London SW1Y 4TQ. (719)

UNIVERSITY OF SYDNEY

POSTDOCTORAL FELLOWSHIP— SCHOOL OF CHEMISTRY

The appointee will work on natural product chemistry (isolation, structure determination and synthesis) under an A.R.G.C. grant. Applicants should have a Ph.D. degree or be about to be awarded one.

Salary within range: $A7,270 to $8,413 per annum. Appointment to commence April/May 1974 and to be for one year in the first instance.

Applications, giving details of qualifications and experience and names of two referees should be forwarded as soon as possible to the Registrar, University of Sydney, New South Wales 2066, Australia. Further information available from Professor E. Ritchie and Dr. W. C. Taylor, Department of Organic Chemistry, University of Sydney.

(775)

UNIVERSITY OF EXETER

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a postdoctoral fellowship for work on the biosynthesis of Bisbenzylisoquinoline Alkaloids, and on related Phenol Oxidations.

Appointment will be for two years commencing as soon as possible. Salary on the scale £1,929-£2,058-£2,223 per annum with placements according to experience. The post is superannuable (FSSU).

Applications, with the names of two referees, should be sent to Professor K. Schofield and Dr D. H. G. Crout, Department of Chemistry, The University, Exeter EX4 4QD, by March 1[illegible] 1974. (588)

STUDENTSHIP IN FISHERIES AND OCEANOGRAPHY

Department of Oceanography

University College, Galway

A studentship is available in the Department of Oceanography for up to 3 years, in the sum of £1,000 plus £500 for expenses, per year, commencing preferably in April, 1974. The applicant needs an M.Sc. degree (preferably in Zoology) and should expect to proceed to the Ph.D. The investigation should explore relationships between occurrences of commercially exploited marine fish, molluscs and crustaceans, and oceanographic conditions. It is expected that research will be necessary into fisheries and oceanographic records and literature in Ireland and elsewhere, and assessment of trends over recent years in catches relative to oceanographic changes about Ireland. There will be opportunities for original research at sea and in the laboratory in connection with the investigation.

Please apply to: **The Head,**
Department of Oceanography,
University College, Galway,

sending your Curriculum Vitae (including passport-sized photo) and names of three associates from whom confidential references may be obtained.

Applications will close on Friday, March 1, 1974. (711)

GRANTS & SCHOLARSHIPS

IMPERIAL CANCER RESEARCH FUND

Bursaries for Training in Research

The Council of the Fund will award a limited number of Bursaries at the Laboratories in Lincoln's Inn Fields and Mill Hill for full-time studies leading to Higher Degrees in research fields relevant to cancer, including Cellular Biology, Endocrinology, Virology and Macromolecular Chemistry.

The award will be tenable for three years from October 1, 1974, with a non-superannuable grant of £1,270 a year (subject to tax), with, in some cases, additional allowances.

Applications are invited from British subjects, not over 25 years of age, normally resident in the UK, who hope to have First or Upper Second class degrees in Science in 1974.

Further details and application forms are obtainable from: **The Personnel Officer, Imperial Cancer Research Fund Laboratories, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX.** Applications to reach the Personnel Officer at the above address not later than March 22, 1974.

(683)

UNIVERSITY OF ABERDEEN

FACULTY OF MEDICINE

MEDICAL ENDOWMENTS RESEARCH STUDENTSHIPS

The Faculty of Medicine has established two Research Studentships paid from its medical endowment funds which will be tenable for three years from October 1974. The value of the Studentships is £695 per annum, with payment of tuition fees and other allowances.

Applications are invited from recent first or upper second class honours graduates in a biological science subject and those who expect so to graduate in summer 1974. The successful candidates will undertake research for the degree of Ph.D. on one of the following projects:

in the Department of Physiology:
(a) Studies on the relationship between synaptic transmitters and mental illness.
(b) A study of the high pressure nervous syndrome.
(c) A study of epilepsy using the seizure sensitive gerbil as a model.

in the Department of Pharmacology:
(d) The effects of the tranquillizer diazepam (valium), and of lithium on neurotransmitter release and enzyme activities of brain nerve terminals.

Further particulars of the Studentships and the projects may be obtained from N. R. D. Begg, Assistant Secretary (Academic), University of Aberdeen, Marischal College, Aberdeen AB9 1AS, to whom applications should be submitted by March 19, 1974. (778)

LECTURES AND COURSES

THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY

POSTGRADUATE COURSE IN SOLID-STATE ELECTRONICS

(M.Sc. and Ph.D.)

A twelve-month postgraduate course in Solid-State Electronics will be held in the Institute commencing in October each year. The aim of the course is to give graduates in physics, electrical engineering or related subjects a thorough background of general solid-state physics, together with a detailed knowledge of those properties of solids which are of importance in electronics, and an introduction to the specialised techniques involved in the manufacture of devices.

Subjects to be covered include Semi-conductors, Dielectrics, Magnetic Materials, Superconductors, Preparation and Purification of Materials, Design of Solid State Devices (Diodes, Transistors, Integrated Circuits, Lasers, Ferrite Cores, etc.).

The course comprises two terms of lectures and laboratory instruction, leading to a written examination at the beginning of the summer term. Candidates for the degree of M.Sc. subsequently undertake a research project on which a dissertation is normally submitted in October or later. Suitable candidates may be given the opportunity to proceed to Ph.D. work and enrolment on the M.Sc. course is the usual method of entry to Ph.D. research in this field.

The course is recognised by the Science Research Council and has an allocation of their studentships.

Further information may be obtained from Professor A. C. Rose-Innes, Department of Electrical Engineering and Electronics, U.M.I.S.T., Manchester M60 1QD. (785)

UNIVERSITY OF LONDON

M.Sc. DEGREE IN RADIATION BIOLOGY

Applications are invited for admission to a one-year full-time Course in Radiation Biology, leading to the M.Sc. degree of the University of London. A two-year part-time Course may also be offered depending on the number of applicants. These Courses will commence in mid-September 1974 and will be organised by the Medical Schools of Guy's, The Middlesex, Royal Free and St Bartholomew's Hospitals. Applicants must hold an honours degree in physical or biological sciences, or an appropriate medical qualification.

Further information and application forms may be obtained from Dr B. S. Mawhinney, Department of Medical Physics, The Royal Free Hospital School of Medicine, 8 Hunter Street, London WC1N 1BP. It is expected that Advanced Course Studentships will be available for suitably qualified candidates.

(706)

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	GMP-PCP, Cat. No. 15843

Comparison of the P-X bond distances and P-X-P bond angles between the β and γ phosphate groups of AMP-PNP, AMP-PCP and ATP is of particular interest.[1]

This X-ray analysis shows that AMP-PNP has retained similar structural charateristics to ATP. The enzymic cleavage by myokinase, hexokinase and ATPases is inhibited, whereas the adenyl cyclase from plasma membranes converts AMP-PNP to cyclic AMP and PNP.[2]

Thus membrane bound ATP utilising enzymes could possibly be distinguished using this inhibitor.[3]

References:

1. M. Larsen, et al., Science, **166**, 1510, (1969).
2. R. G. Yount, et al., Biochemistry, **10**, No.13, 2484-2489, (1971).
3. S. L. Howell and Margaret Whitfield., J. Histochem, Cytochem., **20**, (11), 873-879, (1972).

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